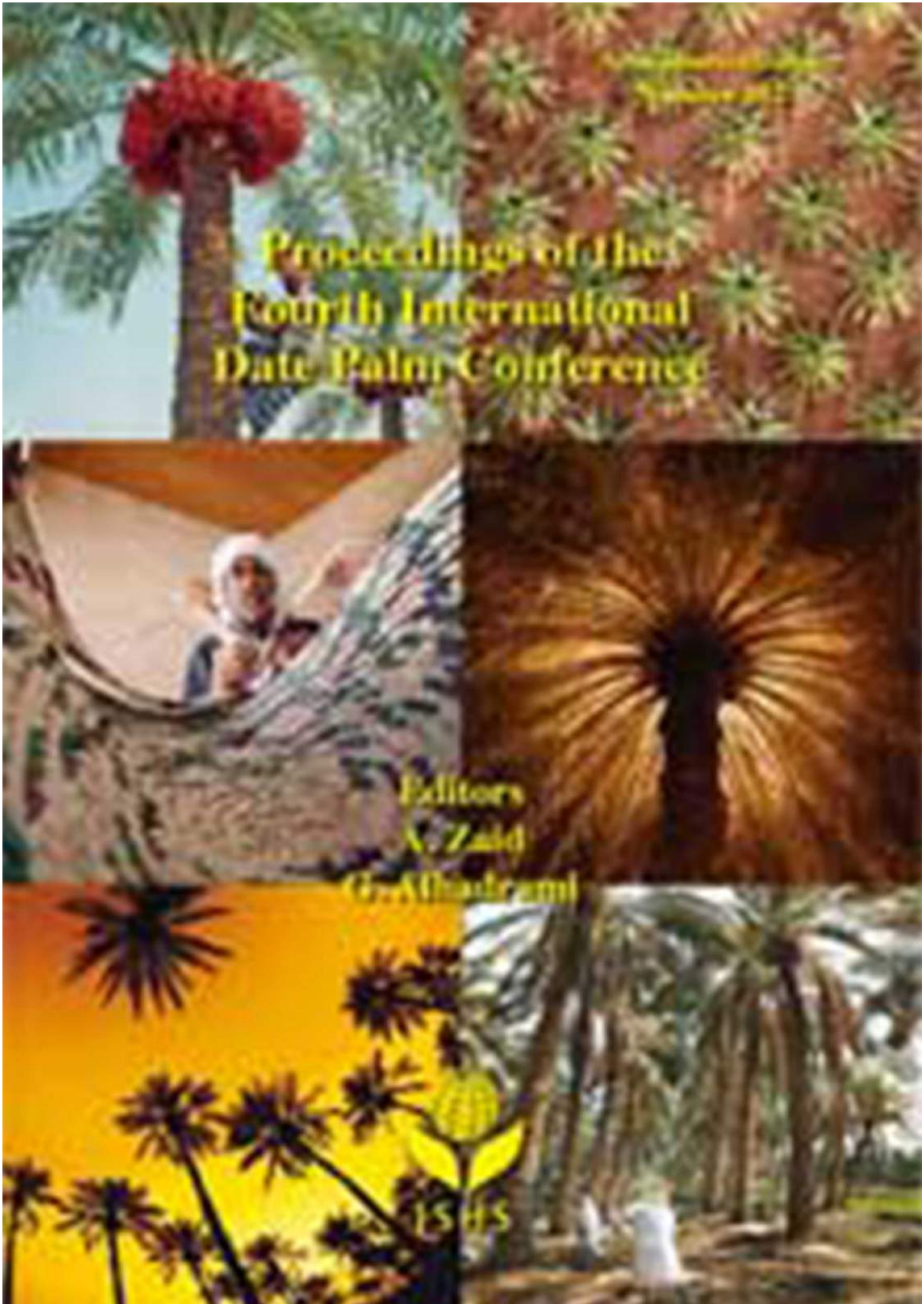
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Development of Date Palm Cultivation and Its Role in Sustainability of Agriculture in Oman

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Abstract

Date palm cultivation is one of the most important agricultural activities in Oman. The date palm is considered as the first important crop in Oman with a perspective to grow. It presents a widespread, integrated ecological and agricultural system. It occupies about 84.9% of the total fruit area and about 49.3% of the total agricultural land. Not only the domestic demand is met, but a significant surplus for export is generated. Tremendous development has occurred in the production and the distribution of date palm during the last three decades. Date palm trees occupy around 84,500 feddan (35,000 ha), and include more than eight million trees.

Since the 1970's, the Ministry of Agriculture has attempted to improve the production of date palm through agricultural research, extension programs and financial support, sustaining various aspects including establishment of tissue culture laboratories with the main objective of providing date palm transplants (offshoots) and preservation of natural resources. Part of the task of the the overall support gene bank is to maintain the most elite Omani date palm. The total production of dates has increased from 173,000 ton in the year 1993 to 298,000 ton in 2001. Environmental constraints such as the limited water resources and the wave of drought that affected the Sultanate in 2002 and 2003 has led to a decline in the date production to 219,772 ton. Yet in 2004-2005 and until 2008 the production of dates has increased to up to 265,000 ton. The development in date production is due to many factors; one of the most important factors is the implementation of new technological methods, including fertilization, insect control, Integrated Pest Management (IPM) and disease as well as the start of a strategy for long term development of date palm in Oman.

INTRODUCTION

Date palm trees (*phoenix dactylifera*) can be short-lived to about 100 years and more. Usually they start fruiting after 3-4 years after planting, up to the top of palm production in the second decade of life after (10-15) years of cultivation, depending on the variety and service operations and environmental conditions surrounding the contribution and continuing at the same rate to the age of 50 years.

The palm tree has radical depth, and a root length of up to 10 meters, which helps to bear the drought, salinity and contributes to the maintenance of the soil from erosion. Because of the features mentioned above the palm tree plays a very important historic role in the Arabian Peninsula, Iraq and some other Arab countries. It is one of the most important economic trees in the hot and dry climate. Also, palm tree is one of the great symbols of the early civilization of Mesopotamia and the Nile Valley. There are differences in the views of historians to clarify the original home of the palm. One opinion shows that the Arabian Gulf is home to the palm and then it was transferred to Iraq some four thousand years BC, where the code of Hammurabi devoted a number of articles for the protection of date palm cultivation, as mentioned in all divine books stated in the Koran as well as in the Hadith.

Date palm is considered the first and most important crop in the Sultanate of Oman, more widespread, and with an agricultural eco-system integrated with great importance in the life of the Omani people throughout the ages as well as wealth and time

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affecting the fabric of Omani in all the words and it touches people's live directly and indirectly. And great efforts are made, especially by the Ministry of Agriculture, to maximize economic returns and social and environmental impacts of palm trees.

Date palm cultivation has expanded and is accompanied by technical and scientific progress which used all its possibilities to find everything new for service in the agricultural sector in general and for the date palm in particular.

Agricultural Land and Production of Food crops for the Sultanate of Oman from 2000 (base year) to 2008: "The results of detailed soil surveys carried out by the Ministry of Agriculture and Fisheries indicated the presence of more than 2.3 million hectares of arable land in the Sultanate. However, the size of the cultivated area is in fact 61,536 hectares of which 42,921 hectares are with perennial crops and 18,615 hectares are with annual crops. Seasonal fruit crops occupy the first rank of the total cultivated area in Oman with 37,000 hectares of which 35,471 hectares of which 10,735 hectares are with field crops under crop rotation and sequence which would raise cropping intensity to the extent of 120%" (Table 1).

DISCUSSION

Date Palm Production

Figures 1 and 2 show the total production of date in Oman for the period from 1994 to 2008 and the index of date production (base year 1994-100).

The production of dates increased from 173,000 tons in 1994 to 281 and 298 thousand tons in 1999, 2000 and 2001, respectively. However, there was a decrease in date production in 2002 and 2003 due to successive drought spells and inaccessibility and lack of rains but productivity in Oman gradually increased year after year until it reached to 267 thousand tons in 2008. The index of date productions was 154 thousand tons. So, the increase in date production was 54 over year 1994. Despite the decline in cultivated area the productivity per palm has been improved. This improvement was due to several factors as discussed later.

From Table 2, the Al-Batina region is considered the leading with 111.4 thousand tons, representing a date production reaching 41.7% of the total output of the Sultanate followed by Al-Dakhilya (20.42%), Al-Sarqiyah (18.32%), Al-Dhahira and Buraimi (13.26%). Other regions are the highest with average productivity per palm (57.22 kg) followed by Al-Dhahira (49.29 kg) and Al-Batina (39.35 kg).

The total number of date palm trees currently has been estimated to around eight million with 700,000 in grading. So the total is 8,700,000 trees with a wide range of available varieties, about 250. The most important characteristics of these varieties are the considerable variation in the quality and maturity dates (early, intermediate, late), the difference in productivity and the rate of annual growth and resistance to pests and diseases. The FAO (1982) reported an estimated annual production of Omani dates of 50,000 tons and the number of date palm trees was 1 million for the period 1961 to 1978. Currently, the date palm trees are estimated to be higher than before due to introduction of new easier production practices along with new cultivars which have increased the large scale farming of date palm. The number has increased to 8,700,000 trees.

The estimation of human consumption for date during the year 2008 was 134,000 tons according to an estimated censuses of Oman in the middle of 2008. About 1,958 million people consume yearly in average 60 kg each; while 821,000 expatriates consume 20 kg each yearly. The amount of dates consumed by animals is about 55,000 tons. The statistics of the Ministry of Commerce indicate that the export of dates in the last year was about 7 tons (Table 4).

Important Constraints in Date Palm Production

The maximization of date palm productivity in Oman is constrained by several factors that include environmental (biotic and a biotic) and agronomic factors, as mentioned below:

- The limited water resources.
- Low soil fertility.
- Small sized farms.
- Low quality of date palm varieties.
- Inexperienced laborers who work at the farm.
- Newly emerged pests and diseases.
- Soil and water salinity.
- High density plantations.

The work accomplished by the Ministry of Agriculture: the research activities in date palm were started in 1995 towards:

- Identification of varieties.
- Better management of different date palm trees varieties in respect to:
  - Water requirement.
  - Irrigation levels.
  - Water use efficiency.
  - Fertilization requirement.
  - Method of pollination, thinning, advancing maturity and pruning.
  - Maintaining the national heritage through conservation of date palm genetic resources.

Factors That Helped the Development of Date Palm in Oman

The generosity of the Almighty God in the palm crop is mentioned in the Holly Qoran and this was the reason why the Omani transported dates across the sea to other markets in Asia and Africa.

The infrastructure needed for agricultural research was completed by establishing two main laboratories:

- The tissue culture laboratory in 1992 (Jimah research Station) in Interior Region.
- The biotechnology laboratory in 2000 at Rumais.

For the purpose of mass propagation of date palm, a genetic map and characterization of Omani date palm genetic resources were established.

The date palm research station in Wadi Quriyat was established in 1988 for the purpose of producing offshoots for replacement and improvement of old varieties. This includes 5000 palm trees, with a distinguished gene bank consisting of 167 female varieties and 20 male varieties carefully selected from all regions of the sultanate.

Another gene bank has been created for palm in the Al-Sarqiyah region for the same reasons mentioned above.

A national strategy to promote date palm and an operational plan of action were developed. The scope of this strategy is to maximise the economic returns, water, social and environmental benefits for cultivation of date palm in the Sultanate on the individual and national level. The most important objectives of this strategy are:

- Production of high quality dates suited to the nature of the consumer as table dates or manufacturing dates.
- Marketing of the production locally and externally throughout the year and finding appropriate manufacturing methods.
- Maximizing the yield potential of palm tree and reducing the cost of production.
- Prevention of the postharvest losses due to diseases and pests in the date palm.

This strategy has the scope to achieve most of its objectives through the implementation within the four main programs:

- Development program for the advancement of productivity.
- Development program for the advancement of marketing.
- Extension program for development date plum.
- Development program for the advancement of research in date palm production.

The first program includes four significant projects:

- Project for propagation and dissemination of the superior varieties (replacement and

reorganization). The objective of this project is to replace 800,000 palm trees which have deteriorated in productivity and old table dates by new and highly productive and qualitative table dates.
- Project of increased yield potential (agronomy).
- Project of integrated control of Red Palm Weevil.
- Project of integrated control of Dubas Bug and other pests.

The second program includes five projects:
- Development project of dates packing units.
- Development project of the manufacturing of dates.
- Development project for manufacturing units for the waste of dates.
- Palm tourism development project.
- Project for date products marketing.

The third program includes three projects:
- Development of a project of quality control specifications for the products of dates.
- Project of replacement the old varieties of date plum trees with new distinguished varieties.
- Deployment project for mobilization of modern dates.

The fourth program includes eight projects:
- Project to study the characterization and evaluation of the Omani varieties and the optimum use for them.
- Project to determine the factors to help increase date plum tree productivity.
- Project of studying the reduction of dates before and after the harvest.
- Project of studying the best methods to control pests and diseases.
- Project to produce tissue culture for distinguished varieties and study distinctive characteristics of date plum.
- Project of food and feed industries of the fruits of palm.
- Project to improve the methods of drying, packaging and manufacturing dates.
- Project to study the best ways to store dates, pollen grains and rutabes.

Date palm cultivation has expanded and is accompanied by technical and scientific progress which used all its possibilities to find everything new in service of the agricultural sector in general and the date palm in particular.

CONCLUSIONS

The date palm is considered the most important fruit crop in the Sultanate of Oman and occupies nearly 50% of the cultivated land in Oman, the larger part of which is in the Batinah region. At present Oman has more than 8 million date palm trees. There are many constraints in agricultural production in Oman, one of them is water scarcity, and the area which is devoted to date palm starts to decline. Also, the farms are small. However, the Ministry of Agriculture is working to maintain this tree in the top of its priority by conserving its genetic resource, carrying out research as well as building appropriate laboratories and expanding the tissue culture lab to increase its productivity of offshoots to 50,000 or more yearly in order to develop the cultivation of this crop and increase the number of date palm trees between 9 to 10 millions. Also, there is tremendous work to control pests and diseases, also to develop an extension service program for Omani date palm growers.

I suggest that the private sector in collaboration with the government give more attention to develop date palm industry through studying the local, regional and international markets. This will help the international stockholders to enhance the current date palm strategy.

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Tables

Table 1. Area (Faddans*) and production (tons) of food crops from 2000 (base year) to 2008 (Source: MoA, 2009).

Year	Crops	Vegetables	Field crops	Forage crops	Fruit crops	Total
2000	Area	15694	14719	42559	100345	173317
	Production	151727	24842	692204	344982	1213755
2005	Area	12267	18192	33101	87884	151444
	Production	119138	26561	539839	307398	992936
2006	Area	11197	18192	34215	87884	151488
	Production	108055	25206	564310	313065	1010635
2007	Area	13207	16952	36515	88255	154930
	Production	130360	25182	608743	311769	1076055
2008	Area	14162	16284	40217	88255	158917
	Production	141073	24572	662503	327628	1155777
% Self sufficiency (Av.)		70%	1%	<85.0%	81%	-

* 1 Faddan = 4200 m^2.

Table 2. The most important area of date palm crop cultivation and production in the Sultanate (thousand tons).

Region	Production	Percentage
Al-Batina	111.4	41.7%
Al-Dakhilya	54.5	20.42%
Al_Sarqiyah	48.9	18.32%
Al-Dhahira and Buraimi	35.4	13.26%
Other regions	16.70	6.30%
Total	266.9	100%

Table 3. The ten most important productive cultivars of date in Sultanate**.**

Cultivars	Year 2007	Year 2008
Um Salia	35465	35218
Mabisily	29698	31175
Khasab	27944	27181
Naghal	25069	24944
Faradh	18956	20482
Shahal	12258	12602
Khalas	12134	12658
Khaneizi	1135	11264
Madluki	4896	5152
Barni	4852	5056

Table 4. The exploitation of local production data (thousand tons) in Oman for the last two years (2007 and 2008).

Year	Year 2007	Year 2008
Total local production	261	267
Human consumption	132	134
Animal feed	53	55
Export	9	7
Surplus of table dates	30	27
Surplus (manufacturing date)	37	46

Figures

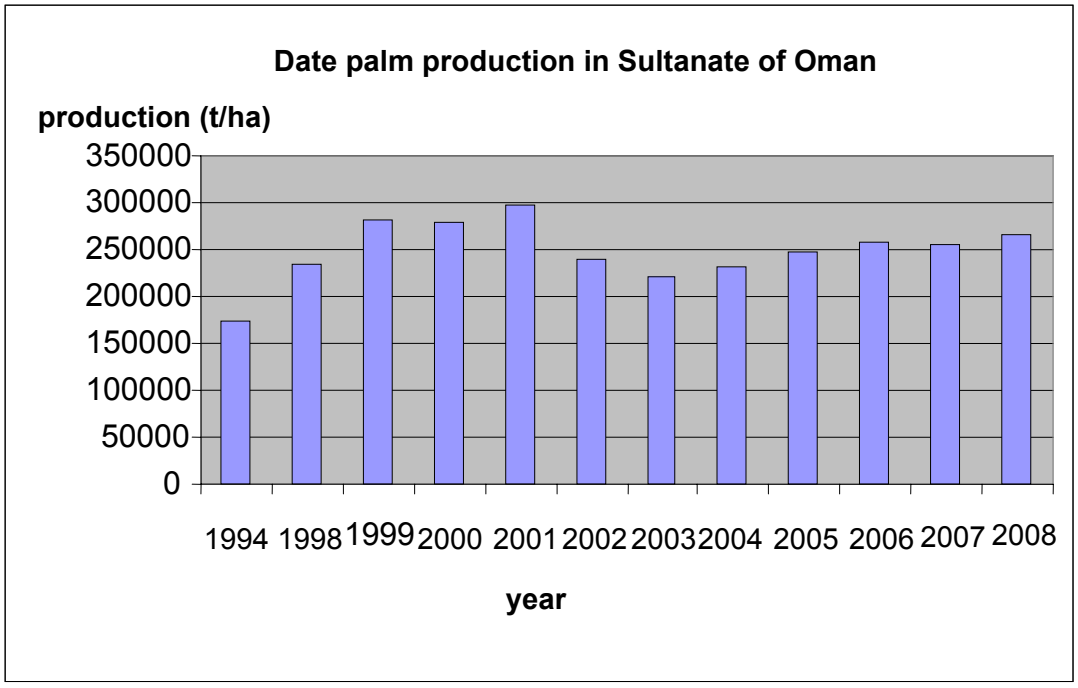

Fig. 1. Date palm production in Sultanate of Oman from 1994 to 2008.

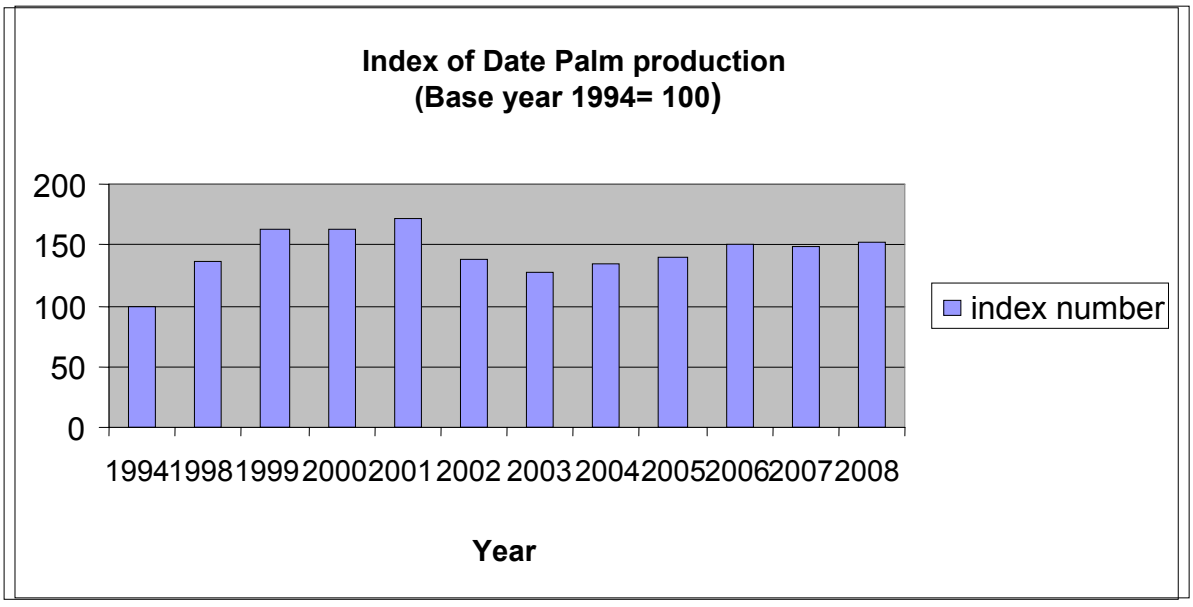

Fig. 2. Date palm production index from 1994-2008 (base year).

The Status of Date Palm Cultivation and Date Production in Sudan

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Abstract

With an annual production of about 330,000 tons and a date palm (*Phoenix dactylifera* L.) population of about 8 million, Sudan ranks number 8 in the list of top date producing countries of the world. However, Sudan has tremendous potential to rank much higher in this scale due to extensive stretches of land between latitude 12°N and the Tropic of Cancer, availability of irrigation water and a suitable climate for date production. Traditionally accustomed to live on date palms by merely pollinating and harvesting the palms, growers in Sudan have to cope with environmental changes and adjust to adopt proper management practices to earn a decent income from date palm cultivation. Sudan has been relying on growing indigenous varieties of dry and some semi-dry dates but the past few years have seen an influx of highly reputed date varieties imported from the tissue culture laboratories in UAE and Saudi Arabia. Research programs on date palms in Sudan are progressing with focus on local selection for promotion of promising indigenous germplasm, male selection studies, propagation, protection, storage and cultural practices. Some efforts to utilize date palm parts in light industry have started but large scale enterprises are yet to come. An overall improvement in harvest, postharvest handling and preparation of dates for marketing in Sudan are required. Sudan is yet free from the devastating Red Palm Weevil (*Rhynchophorus ferrugineus*) Oliv, but termites (*Microcerotermes diversus*, *Odontotermis classic*) Sjosted, white scale (*Parlatoria blanchardii*).Targ., greater date moth (*Arenipsis sabella hampsim*), dust mites (*Oligonychus afrasiaticus*) McGregor, and (*O. pratensis*). Banks and some rodent pests are endemic. The store pests Raisin Moth (*Ephestia* sp.) and the Grain Saw Beetle (*Oryzaephilus surinamensis*) cause a lot of damage. The recently brought in Green Scale (*Astrolecanium* sp.) is a menace in Sudan probably due to lack of predators, vulnerability of local cultivars, climate and lack of growers' awareness to handle an exotic pest. Sudan is yet free from the destructive Bayyoud disease caused by the fungus (*Fusarium oxysporu albedinis*). Black scorch (*Thielaviopsis paradoxa* j.), Graphiola leaf spot (*Graphiola phoenicis*) and inflorescence rot (*Mauginiella scaettae*) are known to exist. The organisms *Fusarium moniliforme, Fusarium oxysporum, Aspergillus* sp. and *Helminthosporium* sp. were isolated. Nematodes have also been isolated from infected date palms. But, several endemic diseases that are known by local names only exist, awaiting a thorough survey to diagnose and identify these diseases.

INTRODUCTION

Area and Production

Date palms *(Phoenix dactylifera)* are intimately linked to the culture, history, heritage, religion and everyday life of the people of northern states of the Sudan. FAO (Stat, 2005) statistics indicate that date palm population in Sudan is about 8 million and that date production is about 330,000 tons. This puts Sudan about number 8 in the list of top date producing countries of the world. Current date palm plantations are mainly strips along the Nile banks north of Khartoum and pockets in Kassala and the Red Sea in the east, and Kutum in the west. But Sudan is a vast country with tremendous potential for expansion in date palm areas. Stretches of flat land from latitude 12°N to the Tropic of Cancer across the entire width of the country is suitable for date production. Expansion in

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date palm areas of Sudan has been slow until tissue culture laboratories availed huge amounts of date palm plantlets of most outstanding date varieties of the world. Sudan benefited from this source and the past two decades were an era of influx of thousands of tissue culture propagated date palm plantlets into the Sudan (Fig. 1). A big portion of these introductions was planted in Khartoum area where growers have the capability to purchase these relatively expensive imported tissue culture propagated plantlets.

There are 5 national tissue culture labs in the country working to propagate date palms, yet the country still relies on imports of date palm plantlets.

Variety Improvement

For centuries, Sudan's date production relied on the six commercial varieties 'Barakawi', 'Abattamoda', 'Gondaila', 'Madeena', 'Mishrig Wad Laggai' and 'Mishrig Wad Khateeb', with the dry variety 'Barakawi' dominating the area. Several seedling cultivars also exist, some of which are known to have very desirable merits. Tissue culture propagated imports from labs in the United Arab Emirates and Saudi Arabia have brought in highly reputed varieties of date palms which include 'Barhee', 'Khalass', 'Abu-Maan', 'Anbara', 'Fard', 'Nubboot saif', 'Nubtat Sulatan', 'Khinaizy', 'Khudry', 'Sukkary', 'Rizaiz', 'Saqae' and 'Sultana'. Early introductions of these varieties are producing well and are being marketed locally (Fig. 2). These introductions are also envisaged to change the nature of Sudan's predominantly dry dates to softer dates which are more palatable and appealing to consumers. A switch in this trend is also envisaged by influence of a new dam which was recently constructed on the River Nile at Mirwy in the heart of the date production area. This dam is envisaged to modify the micro-climate of the area and avail a more humid atmosphere that will soften the dry dates. It may also avail a reliable and cheaper source of electric power that may facilitate cheaper means for date palm irrigation, rutab cold storage and power for packaging and processing factories. The variety improvement program in Sudan also includes surveys to locate candidate date palms with outstanding merits for evaluation, selection and promotion of the best. The authors are evaluating a wide selection of these cultivars for local selection (Fig. 3).

Male Selection

Despite the existence of a prehistoric date palm culture in Sudan, no named males exist and commercial date palm growers still depend on random males for pollen collection. Yet date palm growers in Sudan realize that some difference in pollen effects can be observed and growers evade taking pollen from certain male palms. The only named males are probably 'New Halfa 1' and 'New Halfa 2' which were recently released for pollinating 'Mishrig Wad Lagai' and 'Mishrig Wad Khatib', as well as the recent tissue culture propagated male introductions. Realizing the need to work for male selection, the authors are evaluating some local males for selection and multiplication. Diverse xenia and meta-xenia effects were detected, emphasizing the need to pursue these studies.

Cultural Operations

Date palm culture in Sudan is still traditional, apart from few plantations where implantation of recent techniques are being examined. Multiple stemmed clumps due to lack of desuckering are common, resulting in crowded offshoots that harbor rodents, impede cultural operations and reduce the quality of dates. Dry fronds are not properly removed. Leaf bases, which are sometimes weak and infested by pests, are used for getting to the tops of date palms. As a result, occasional incidents of injuries and tragic deaths of people falling from date palm tops may occur. In collaboration with Iraqi expertise, efforts to improve the techniques of climbing date palms using safety ropes like neighboring countries are in progress (Fig. 4).

No bunch management techniques are practised apart from a few trials in recent plantations. Harvest, handling, storage and means of date display for sales need to be improved.

Packing and Processing

There is one government date packaging factory in the country. Some private companies process medical alcohol and vinegar from dates. But these operations need to be improved by construction of more modern plants to cope with the expanding date production.

Utilization of Date Palm Parts

There was a time when date palm parts were extensively used in construction of houses, water wheels for pumping water off the River Nile, containers, mattresses, ropes, fencing, fire wood and the like. While traditional means of utilization of date palm parts continue, means of utilizing date palm parts is expanding to include manufacturing furniture, beds and innovative products (Fig. 5).

PROTECTION

Pests

Fortunately, Sudan is yet free from the devastating Red Weevil pest (*Rhynchophorus ferrugineus* Oliv). Strong prohibitive measures to restrict its entrance into the country are effective to date. Indigenous pests the most important of which are termites *Microcerotermes diversus*, *Odontotermis classic* Sjostedt, white scale *Parlatoria blanchardii.*Targ., greater date moth *Arenipsis sabella hampsim*, dust mites *Oligonychus afrasiaticus* McGregor, and *O. pratensis.* Banks exist in the country and control measures continue. Store pests like raisin moth (*Ephestia* sp.) and the grain saw beetle (*Oryzaephilus surinamensis* L.) cause serious damage to dates and proper care from the palms to consumers is undertaken. Rodents like mice, rabbits, birds such as house sparrow (*Passer domesticus* Arbreus) and bats are endemic and cause a lot of damage. The exotic green scale *Asterlecanium*, which is relatively a new pest in Sudan, poses a real threat to the date wealth of Sudan (Fig. 6). This pest is devastating and control measures are very expensive. Chemicals, IPM and burning have been tried but the final control measure seems to be biological control. The reason why green scale which is not considered to be a serious pest in countries where it is endemic, while so serious in Sudan, could be because there are no effective predators in Sudan, or because some Sudanese varieties are more susceptible to the pest attack or the availability of a more favorable environmental factor. Further, there is a lack of technical knowhow in handling an exotic pest. With failure of strong quarantine measures to confine the pest to the spot of its first appearance and effective demolishing measures, it has not been possible to eradicate the pest completely. Since the pest is widespread now, current control measures are IPM, sanitary, coupled with chemical control and adoption of plant quarantine legislations. Predators for biological control are being introduced from areas of similar habitats in Saudi Arabia and Iran for breeding and release in green scale infested areas.

Diseases

Sudan is so far free from the devastating Bayyoud disease (*Fusarium oxysporum albedinis*) but several diseases, many of which are only known by local names exist. Black scorch (*Thielaviopsis paradoxa* j.), inflorescence rot (*Mauginiella scaettae*) and Graphiola leaf spot (*Graphiola phoenicis*) are known to exist. The organisms *Fusarium moniliforme*, *Fusarium oxysporum*, *Aspergillus* sp. and *Helminthosporium* sp. were isolated. Nematodes have also been isolated from infected date palms. A thorough survey to diagnose and identify the endemic diseases of date palms in Sudan awaits investigation

Closure

The prospects for establishment of an advanced date industry in the Sudan are enormous, vast areas with suitable climate and irrigation facilities are available. Infrastructure and human resources with high technical capabilities also exist. With current availability of date palm plantlets from tissue culture laboratories, boosting the

date palm area with highly esteemed date varieties are in progress.

Collaboration

Intimate regional collaboration to develop the date palm sector in the region and improve the livelihood of date palm growers is extremely vital. Relevant areas for collaboration are research work, propagation, date palm service improvement, postharvest handling, and packaging, processing and marketing. Establishment of regional institutions to facilitate such linkages and exchange of experience is crucial to achieve the ultimate goals. The long time dream of establishing an international palm and date centre waits to become true.

Figures

Fig. 1. Elshamil Nurseries-Kadaro, Sudan: torpedoes and advanced date palm plantlets.

Fig. 2. Bearing Barhee- Elnifaidy Orchard, Soba, Khartoum.

Fig. 3. Candidate date palm cultivars for local selection (Merowe, Sudan).

Fig. 4. Training nationals in removal of date palm leaf bases and climbing by ropes.

Fig. 5. Furniture manufacturing from date palm parts (Curtsey of Muthanna K. Chechani).

Fig. 6. Green scale on date palms - Artigasha Island, Sudan.

Potential of Date Palm Plantation in Afar Region, Ethiopia and Its Market

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Abstract

The Afar Region in Ethiopia is home to a vast area of virgin land that is suitable for date plantation. The region is located within the Danakil depression and experiences the harshest climate in Ethiopia with temperatures reaching up to 50°C. The area is occupied by the Afar people who are primarily semi-nomadic. Historically the Afars have established date farms in areas around the Awsa delta and Afambo region. These farms produce date fruits mainly for local consumption. The main source of irrigation in the Afar Region is from the Awash River. The Awash River runs from the Ethiopian highlands, fed by a number of tributaries, through the Afar desert land. Producing date fruit in the Afar region will have a mutual benefit for both the investor and the local population. There is currently a shortage of supply for date fruit in Ethiopia where the demand for the produce is at its peak during the month of Ramadan. Current statistics show about a third of the Ethiopian population to be muslims, creating a large market for date fruits locally. Labour cost in Ethiopia is significantly low compared with date producing countries such as the United Arab Emirates (UAE). It is therefore attractive to produce locally at lower cost to meet this high demand throughout Ethiopia and possibly beyond. Date fruit contains essential vitamins and minerals that are required for a balanced diet. Setting up date plantations in this region in sufficiently large quantity will ensure a source of date fruit for the local community. This has the potential to improve the health and food security for the nomadic Afar community. Jobs that will be filled by local farmers will create a source of income and can help develop and transfer the whole community as a whole. Large scale date plantation in this desert land will have a significant contribution towards the fight against climate change.

INTRODUCTION

The purpose of this paper is to assess the potential for a date palm plantation project in the Afar Region of Ethiopia on the Horn of Africa.

The region is geographically located in the North-Eastern part of Ethiopia, bordering with Eritrea to the North and Djibouti to the East.

The land is occupied by the Afar people who live in three adjoining countries of East Africa. These are Ethiopia, Eritrea and Djibouti. On the Eritrean side the Afar people occupy up to 800 km of the Red Sea strip. On the Djibouti side they also occupy all of the costal strips. Historically the Afar people have trade connections with the Arab world as a result of their geographic location.

Islamic history indicates that the first immigration of the Prophet's (SAW) followers to 'Ardul Habasha' went through the Afar regions indicating the historic link that they had with the Arab world dating as far back as 1400 years ago. Date plantation in the region was inherited from the Arabs. The Afar have established date farms in areas around the Awsa in Afambo District. These farms produce date fruits mainly for local consumption.

The majority of the Afar populations in Ethiopia are nomadic, traveling from place to place with their livestock for grazing land. Their main nutritional source is bread and milk.

The following sections present the potential for date palm plantation in the Afar Region by looking at the available land, water source and market.

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DISCUSSION

Land Potential

Based on data received from the Afar Regional Agricultural Bureau (2007/8), the Afar region in Ethiopia covers approximately 9.5 million hectares of land of which 12.6% is cultivatable land. The region has a vast area of virgin land that is suitable for agriculture. The total available land that has agricultural potential but has not been cultivated is approximately 1.2 million hectares.

Out of the above, grazing land constitutes 863,000 hectares of the available cultivatable land, forest land comprises 140,000 hectare and remaining 222,000 hectares is virgin land. At the time of the survey, total cultivated land constituted 75,000 hectares.

In addition, the data show approximately 3.6 million hectare of land that has currently been classified as un-cultivatable land due to its unsuitability for crops. It is anticipated that 10% of this land (approximately 300,000 hectares) will be suitable for date palm plantation. This is mainly because date palm has better tolerance of semi-saline soils compared with other crops. Subsequently out of this land up to 40% (120,000 hectares) has the potential to be cultivated for date plantation by gravity irrigation from the Awash River due to its proximity to the river as it is located in one area between Dubti and Aysaita Districts. The recent construction of the Tandaho Dam, which is under completion, has increased the potential of water resource capacity for gravity irrigation to cultivate partially or in full the aforementioned landmass.

The remaining 60% of land is allocated in small patches across a number of districts. These patches of land could potentially be cultivated using underground water. However, this will require an initial investigation work for underground water.

Water Source

The main water source for irrigation in this region is the Awash River. Historically, a number of large scale plantation projects have been established and irrigated from the Awash River such as the Tendaho plantation project. The River flows from the Ethiopian highlands and passes through the Afar desert land to the Djibouti border. The river is approximately 1100 km long. There are a number of tributary rivers and streams feeding the river along its way. In addition to the Awash River and its tributaries, all the lowlands adjacent to the north eastern escarpment of the Ethiopian plateau particularly Zone 4 and 5 is believed to be very rich in underground water and suitable for underground water harvest.

Market

Ethiopia currently imports date fruits from different date growing countries by paying hard currency. This is because there are no commercial date plantation farms in Ethiopia.

Ethiopia has high demand for date fruit mainly during the month of Ramadan. This is because one third of the Ethiopian population are muslims (approximately 28.9 million people), creating high demand for date fruit during this month and other religious ceremonies and traditional rituals.

According to Ethiopia's capital city Addis Ababa Custom Office Records (2010), the average quantity of date fruit imported to the capital city (Addis Ababa) alone is 19,000 metric tons per year.

Regions which have high population of muslim communities such as Ugaden, Afar and Binishankul are located along the Ethiopian borders. As such they import date fruits from their bordering countries such as Somalia, Djibouti and Sudan respectively. The Harrar region also imports from Somalia and Djibouti. These are mostly unrecorded commodity imports. This shows that the demand for date fruit in these regions is much higher than what is imported to the capital city as they have a higher muslim population when compared with Addis Ababa.

The quantity of date fruits imported to the capital Addis Ababa and the selling

price for the past three years is presented in Figure 3. The graph shows an increase in demand and price. The increase in sales could be due to an increase in population and growth in socio-economic scale. The increase in price is mainly due to the increasing exchange rate between the American Dollar and the Ethiopian Birr. In 2007, $ 4.00 was 35 ETH Birr. In 2008, it was 40 ETH Birr and in 2009 $ 4.00 was 60 ETH Birr. This shows there is no change in the price of date fruit in dollars.

When looking at the market price of date fruit imported through other Ethiopian borders, the regional market price does not have much difference from the central market price. This shows the market price of date fruits is not due to the import duty tax but the slight difference could be due to transportation cost.

When assessing the relationship between demand and price in Ethiopian Birr, it shows that an increase in price does not decrease demand. This shows the product is an inelastic product. This is because the muslim communities view date fruit as 'sunna' during the month of Ramadan to break their fast and they consume it as 'sadaqa' at religious ceremonies and rituals. Therefore, the demand will be as a minimum constant or growing due to these other factors.

When looking at the total muslim population in Ethiopia and the religious significance of date fruit, the total demand should be much higher than the current demand. Assuming 70% of the muslim population consume date fruit and one person consumes 4 kg of date fruit per year. The yearly demand becomes 80,000 metric tons. This is without including the rest 65% of the Ethiopian population (50 million people). Assuming 50% of the remaining 50 million people consume 1 kg/person/year, there is a potential demand for 25,000 metric tons.

This shows that the amount imported is much lower than the potential demand. This is because the cost of importing the fruit is expensive hence the selling price in the market is much more than what even the middle class income category of the population can afford.

The market price of date fruit is currently not affordable to most Ethiopians mainly because date producing countries have high costs of production such as labour cost and irrigation. If, however date fruit is produced in the Afar region in Ethiopia, the cost of labour will be cheaper when compared with date producing countries. There is also a convenient source of water for gravity irrigation.

Benefits of Large Scale Investment in Date Plantation

Large scale investment in date palm plantation will provide food security not only for the immediate local community but also for the whole of Ethiopia. Ethiopia has a long standing problem with famine. Due to its nutritional value, date fruit is very important emergency food. A small quantity can potentially save a large number of people.

Currently, date fruits are mainly imported from other countries. As a result the date fruits are expensive and a lot of people cannot afford to buy it. If large scale plantation is established in Ethiopia, then date fruits can be introduced into the market at an affordable price which the local community can benefit from.

Large scale plantation will create job opportunity to the local Afar people. This will provide the pastoralist Afar people an opportunity to pursue a sedentary life as a result of a constant source of income and sustainable livelihood. It will also help to contribute to the region's economy as well as the country's economy by distributing the date fruit to the rest of Ethiopia and potentially exporting to other countries.

General Benefit of Date Plantation to the Local Community

The people of Afar largely depend on their livestock for food source. The people who live around the river banks have small crop farms. Those who live away from the river banks move from place to place in search of grazing land for their livestock. About 90% of the Afar people are nomads and do not have the necessary knowhow or capability to establish agricultural farms as source of food and income. Currently, a very small group of people are engaged at subsistence agriculture. As a result they suffer from

poverty and food shortage.

Due to its nutritional value, date fruits can be used as a source of food. It can also be stored for a long period of time depending on the type of date fruit. For nomads it is convenient to transport and use.

The other advantage of date plantation is that once established, date farms are low maintenance farms compared with other agricultural farms in that there is no need to cultivate every year. Other farms will require re-planting after each harvest which requires reinvesting. The main problem is that the farmers do not have the knowledge to manage their account to allow them to continue to farm. Date palm on the other hand only requires initial investment. Once established it requires low maintenance work such as watering. The fruit is then harvested every year. It has low operational cost.

Date plants have very high production compared with other farms for a given area of land. This will encourage the people in agriculture if they see that they can get good results from a small effort and secure food for their families.

Impact on Climate Change

The Afar region is located in the Denakil depression and is generally desert land. It experiences the harshest climate with temperatures reaching up to 50°C during dry seasons in the lowest area. The average temperature in the Middle Awash is 25°C and in the Lower Awash 35 to 45°C.

Large scale plantation of date plants will help create forests of trees in this desert land. This will have a positive impact in improving the regions climate and reducing the occurrence of drought locally and will contribute towards the fight against climate change globally.

CONCLUSIONS

In conclusion, establishing a date palm plantation project can benefit both investors and development support organisations such as the World Development Bank, the Islamic Development Bank, UNDP, and FAO are some examples.

For investors, there is a large market available for date fruit in Ethiopia with low cost of production compared with other date producing countries.

Development support agencies can look at the benefits of date plantation to improving socio-economic development of countries such as Ethiopia. It also helps to decrease the date fruit price in Ethiopia by producing it locally and to ensure food security.

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Figures

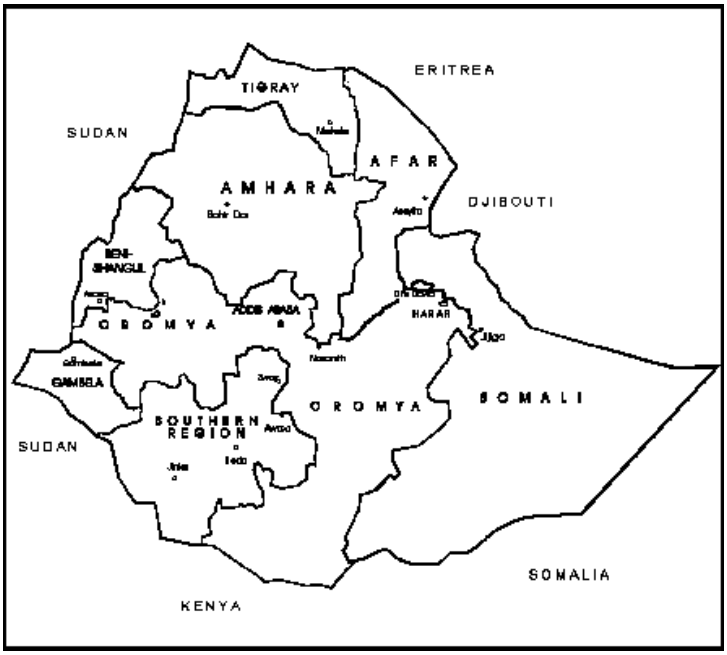

Fig. 1. The Afar Region in Ethiopia.

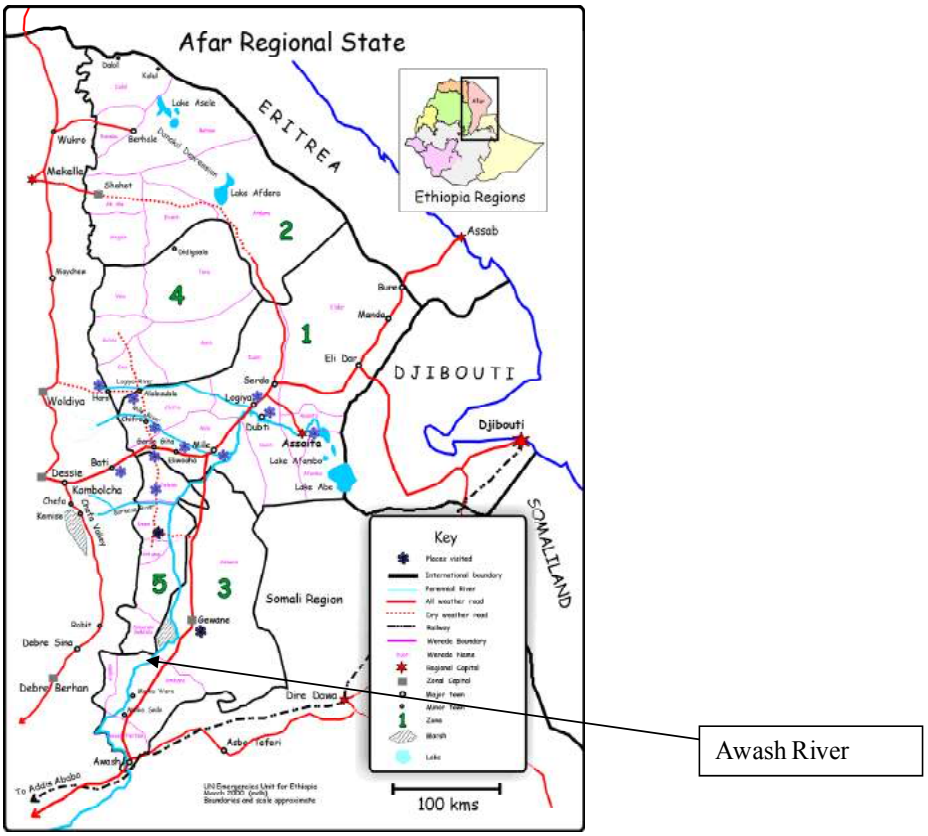

Fig. 2. Map showing the Afar Region and its rivers (image courtesy of the UN).

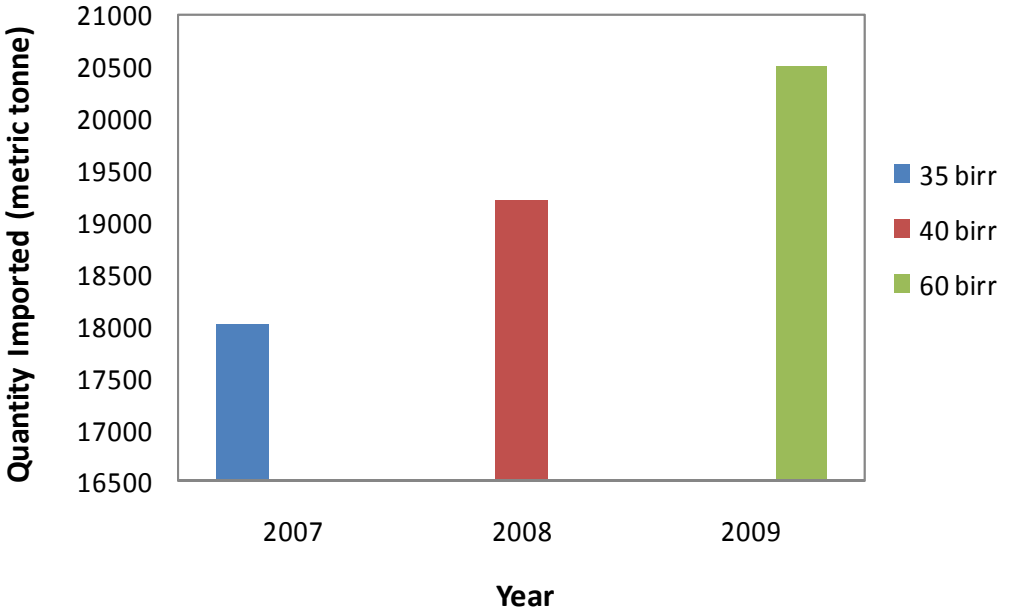

Fig. 3. Imported quantity of date fruit and selling price per kilogram in Addis Ababa.

Inefficiency in the Market Profit Distribution Affected Date Palm Production in Yemen

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Yemen

Keywords: inefficiency in market profit distribution, date palm production, Yemen, farm gate price, producer surplus, consumer price, market margin, middleman marketing profit, fair prices

Abstract

Farmers in Yemen are facing many problems related to natural resource endowments, availability of water and in the marketing of their products. Despite the difficult natural circumstances the farmers produce agricultural commodities, including date palm. This product is sometimes sold in local markets and sometimes the product is sold to middlemen who transport the commodities to the markets or directly to the consumers. The value added in the chain will be distributed over the various actors, the farmers, the transportation sector and the middlemen.

As a result of the actions of the middlemen only a small part of the value added in the chain is to the benefit of the farmers. The prices that are offered to the farmers do not allow compensating for the costs and this results in low profits or no profits at all to the farmers.

As a result, the farmers are not able to expand their activities and are facing very low incomes and the risk of complete bankruptcy. This is a limiting factor for agricultural development and hampers modernization of the date palm trees in Yemen.

Based on the feasibility study of net revenue $/ha for a farm in Wadi Hadramout during 2002 to 2006 the net revenue is negative for the farmers. In 2006 the farmer's loss is 233 $/ha. While the middlemen gains a profit of about 4,256 $/ha, for the same year which means that the net marketing margin is 244% for the middlemen profit. Marketing is frustration for small farmers in Yemen. Farmer's production increased, but their income did not.

METHODOLOGY

This study was based on data collected from the Ministry of Agriculture and Irrigation Yemen, Department of Monitoring and Evaluation, for the cost of Date Palm producers, market intermediaries in production. The retail price of date palm was calculated from Sana'a City market. Wadi Hadramout was selected for this study because it is considered the main date palm growing area in Yemen.

The successful use of advanced methods of marketing analysis is heavily dependent on the availability of data which is not the case in Yemen; secondary data like national statistics and surveys conducted by different organizations. Especially with the latter, it is likely that they have followed different standards and procedures and hence may vary significantly in quality, validity and representativeness. Hence, there is a need for a thorough validation and repeated discussion of the results. Such discussion will yield the need for additional data collection, especially when dealing with specific operational decisions such as intervention targeting and support intensity. In order to empirically advance for this case the existing data were used in the input-output analysis (Tables 1-5 in Appendix) for date palm marketing in Wadi Hadramout.

MARKET MARGIN ANALYSIS

The marketing margin are the differences between prices at two market levels: farm gate price and consumers price. Marketing margins have been examined on the basis

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of data obtained for prices at different stages of the marketing chain. Marketing margins have been calculated through computing the absolute margins or price spread, which is essentially the same as the difference between the prices, paid and received by each specific marketing agency. The following formula has been used to compute percentage marketing margins as earned by each market intermediary in the marketing of farm products.

1. Farm profit = Gross revenue ($/ha) - total costs ($/ha) (Table 2).
2. Marketing margin = Farm gate price - consumer price (Table 3).
3. Percentage marketing margins = Farm gate price - consumer price/farm get price * 100 (Table 4).

Table 2 shows that Farm profit = gross revenue ($/ha) - total costs ($/ha) = 1,744 - 1,977 = -233 $/ha. That means that the farm has received lower prices than the real cost of production. The average sum lost is -806 $/ha (2002 to 2006).

Table 3 shows that middlemen have received high profit. It is 1,109 $/ha in 2002 up to 4,256 $/ha in 2006, with an average period of 2,307 $/ha.

Table 4 shows that the percentage in marketing margins earned by the middlemen in Sana'a is 172% 2002 to 244% in 2006 with an average period 238%

Breakdown of Consumer's One USA Dollar

Breakdown of consumer's dollars is a phrase applied to the manner in which a consumer's dollars expenditure on a particular commodity is divided among the producer and marketing agencies. It shows from Table 4 that the portion of consumer's dollars which goes to the producer is 0.44 cent and 1.06 is earned by various marketing agencies such as contractors, commission agents, wholesalers and retailers. This was calculated by expressing the net margin of a specific agency as proportion of the retail price.

Marketing Costs

The marketing margin indicates the amount received by different marketing agencies for providing their services, from the time when the commodity leaves the farm until it reaches the consumers. Such costs are not known and are not included in the analysis.

CONCLUSIONS

Like farmers throughout the world, but especially in developing countries, Yemeni farmers work hard throughout the year to produce high quality crop and livestock products in sufficient quantities to reach profitable levels. However, also like farmers everywhere, Yemeni farmers lack marketing information, alternatives, knowledge, skills, tools, and institutions to make the most of selling the products they worked so hard to produce. Marketing issues are particularly frustrating for farmers because they often perceive that the 'middle man' or the broker gets more of the consumer dollar than the farmer does. Marketing is frustration for small farmers in Yemen. Farmer's production increased, but their income did not.

RECOMMENDATIONS

It is strongly recommended that policy for equity and normal margin profit for both farmers and middlemen are applying inside the retail market areas in Yemen.

Efforts are needed to improve date palm production in Yemen and link it with food security and poverty elevation.

Agricultural economists at the Faculty of Agriculture, Sana'a University, Sana'a, ROY, in cooperation with UAE University conduct joint baseline surveys to carry out reliable econometric analyses in date palm production in Yemen.

Farmers want new marketing principles to enhanced marketing capacity and policy for small farmers to get fair prices for their production.

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Tables

Table 1. Crop budgets of the main cropping patron in Wadi Hadramout 2006.

Cropping pattern	Average	Dates	Alfafa	Mango	Banana	Onion	Tomato	Garlic	Potato
Gross Revenue($/ha)	4,469	1,744	6,380	7,356	4,905	3,834	-	7,200	4,336
Total costs ($/ha)	1,534.7	1,977	2,265	2,372	2,221	1,150	359	1,060	873
Net Revenue ($/ha)	2,935	233	4,115	4,984	2,684	2,684	359	6,140	3,463

Calculated from Tables in appendix 1.
Table 1 shows that all crops net revenue is positive, except the Date Palm and Tomato are negative by 233 and 359 USA$.

Table 2. Net Revenue ($/ha) for farm date palm in Wadi Hadramout 2002 to 2006.

	2006	2005	2004	2003	2002
Yield (kg/ha)	4,000	2,200	2,080	1,384	1,350
Farm gate price ($/kg)	0.44	0.4427	0.421053	0.34	0.479
Gross revenue ($/ha)	1,744	974	876	473	646
Total costs ($/ha)	1,977	1,959	1,583	1,706	1,521
Net revenue ($/ha for farm)	233	985	707	1,232	875

Calculated from Tables un appendix 1.

Table 3. Net Revenue ($)/ha for middlemen in Sana'a City 2002 to 2006.

	2006	2005	2004	2003	2002
Consumer price (retail price in Sana'a City)	1.5	1.5	1.5	1.3	1.30
Gross revenue($/ha)	6,000	3,300	3,120	2,075	1,755
Total costs ($/ha)	1,744	974	876	473	646
Net revenue ($/ha for middlemen)	4,256	2,326	2,244	1,602	1,109

Calculated from Tables in appendix 1.

Table 4. Percentage marketing margins earned by the middlemen in Sana'a 2002 to 2006.

	2006	2005	2004	2003	2002
Farm gate price ($/kg)	0.44	0.4427	0.42105	0.34	0.479
Consumer Price ($/kg)	1.5	1.5	1.5	1.3	1.30
Market Margin ($/kg)	1.06	1.06	1.08	0.96	0.82
% Market margin ($/kg) for middlemen	244.12	238.82	256.25	280.00	171.56

Middlemen profit = Gross revenue ($/ha) - total costs ($/ha) = 6,000 – 1744 = 4256 $/ha.
From Table 4 the marketing margins earned by the middlemen in Sana'a was (1.06/.44*100) = 244 $/kg (Retail price in Sana'a City).

Table 5. Date palm prices in Sana'a City 2007-2008.

2008 price ($/kg)	Inflation Rate	2008 price (YR/kg)	2007 price (YR/kg)	Kind
3	200	600	300	Al-Ngrani (Saudi)
2.5	167	500	300	Al-Hadhramy(Saudi)
2.75	183	550	300	Al-Bashy(Saudi)

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Table 5 explains the market situation, Retail price in Sana'a City increased from 1.5 US$ in 2007 to 3 US$ in 2008 this means the inflation rate is 200% for date palm. Also, the Family Budget Survey 2008 indicted that Yemen had import date palm from Saudi Arabia by 30 million USA dollar, these indicated demand is higher than the supply.

Appendix

Crop budgets input-output analysis of the main cropping patterns in Wadi Hadramout 2002 to 2006.

Table 1. Crop budgets input output analysis of the main cropping patterns in Wadi Hadramout 2006.

Cropping pattern	Dates	Alfafa	Banana	Onion	Tomato	Garlic	Potato
Area cultivated(million ha)[1]	0.005	0.00321	0.00022	0.00123	0.00025	0.00012	0.00019
Rainfed area (million ha)	0.002695						
Irrigated area (million ha)	0.00270	0.00321	0.00022	0.00123	0.00025	0.00012	0.00019
Irrigation water use (m^3/ha)[2]	19,987	21,640	25,583	12,459	7,032	11,509	6,662
Total irrigation use (MCM)	53.86	69.51	5.63	15.32	1.78	1.36	1.26
Yield (kg)/ha	4,000	11,000	11,964	8,520		4,500	8,672
Farm gate price ($/kg)	0.44	0.580	0.41	0.5		1.6	0.5
Gross revenue ($)/ha	1,744	6,380	4,905	3,834	-	7,200	4,336
Water related costs ($/$m^3$)	0.08	0.08	0.06	0.06	0.05	0.05	0.04
Capital costs ($/$m^3$)	0.043	0.039	0.031	0.028	0.025	0.023	0.021
Maintenance ($/$m^3$)	0.0072	0.007	0.005	0.005	0.004	0.004	0.003
Operation - diesel ($/$m^3$)	0.033	0.030	0.024	0.022	0.019	0.017	0.016
Diesel use (L/m^3 water)	0.18	0.165	0.134	0.120	0.108	0.097	0.088
Cost of diesel ($/L)	0.179	0.162	0.131	0.118	0.106	0.095	0.086
Operation (oil $/$m^3$)	0.003	0.003	0.002	0.002	0.002	0.002	0.002
Labor costs ($/ha)[3]	124	382	397	317		316	378
Other costs[4]	175	197.00	210	125		215	219
Irrigation water applied (m/ha)	2.00	2.16	2.56	1.25	0.70	1.15	0.67
water cost ($/ha)	1,678	1,686	1,614	708	359	529	276
Total costs ($/ha)	1,977	2,265	2,221	1,150	359	1,060	873
Net Revenue ($/ha)	233	4,115	2,684	2,684	359	6,140	3,463
Returns to water ($/$m^3$)	0.1	0.3	0.2	0.3	-	0.6	0.7

[1] Ag. S. Sources of Area and productivity are the Agricultural Year Kook – 2002 to 2006.
[2] Grw. S. P. Groundwater & Soil Conservation Project, Sauyen Hadramout December 2007.
[3] DRC المحلية الموارد تكاليف دراسة) الفاو -خبير العزيز عبد د. ،2006 لعام الزراعي الإحصاء ، البشرية التنمية تقرير :المصادر.
[4] Other costs (manure, urea, chemical fertilizers, chemicals) ($/ha).

Table 2. Crop budgets input output analysis of the main cropping patterns in Wadi Hadramout 2005.

Cropping pattern	Dates	Alfafa	Banana	Onion	Tomato	Garlic	Potato
Area cultivated (million ha)[1]	0.0053900	0.0028900	0.0002100	0.0012000	0.0002460	0.0001200	0.0001800
Rainfed area (million ha)	0.002695						
Irrigated area (million ha)	0.0026950	0.0028900	0.0002100	0.0012000	0.0002460	0.0001200	0.0001800
Irrigation water use(m^3/ha)[2]	20,131	22,590	27,037	13,096	7,389	12,016	6,946
Total irrigation use (MCM)	54.25	65.29	5.68	15.71	1.82	1.44	1.25
Yield (kg)/ha	2,200	13,441	11,948	8,543	8,586	4,522	8,604
Farm gate price ($/kg)	0.4427	0.730	0.43	0.4	0.32	1.23	0.29
Gross revenue ($/ha)	974	9,812	5,138	3,332	2,748	5,562	2,495
Water related costs ($/$m^3$)	0.08	0.08	0.06	0.06	0.05	0.05	0.04
Capital costs ($/$m^3$)	0.043	0.04	0.03	0.03	0.03	0.02	0.02
Maintenance ($/$m^3$)	0.0072	0.01	0.01	0.00	0.00	0.00	0.00
Operation - diesel ($/$m^3$)	0.033	0.03	0.02	0.02	0.02	0.02	0.02
diesel use (L/m^3 water)	0.18	0.17	0.13	0.12	0.11	0.10	0.09
Cost of diesel ($/L)	0.179	0.16	0.13	0.12	0.11	0.10	0.09
Operation (Oil $/$m^3$)	0.003	0.00	0.00	0.00	0.00	0.00	0.00
Labor costs ($/ha)[3]	124	395	373	312	387	316	364
Other costs[4]	145	236.00	205	185	223	177	239
Irrigation water applied (m/ha)	2.01	2.26	2.70	1.31	0.74	1.20	0.69
water cost ($/ha)	1,690	1,760	1,706	744	378	553	288
Total costs ($/ha)	1,959	2,391	2,284	1,241	988	1,046	891
Net revenue ($/ha)	985-	7,421	2,853	2,091	1,760	4,516	1,605
Returns to water ($/$m^3$)	0.0	0.4	0.2	0.3	0.4	0.5	0.4

[1] Ag. S. Sources of Area and productivity are the Agricultural Year Kook – 2002 to 2006.
[2] Grw. S. P. Groundwater & Soil Conservation Project, Sauyen Hadramout December 2007.
[3] DRC المصادر: تقرير التنمية البشرية ، الإحصاء الزراعي لعام 2006، د. عبد العزيز -خبير الفاو.
[4] Other costs (manure, urea, chemical fertilizers, chemicals) ($/ha).

Table 3. Crop budgets input output analysis of the main cropping patterns in Wadi Hadramout 2004.

Cropping pattern	Dates	Alfafa	Banana	Onion	Tomato	Garlic	Potato
Area cultivated (million ha)[1]	0.0053630	0.0028820	0.0001500	0.0005300	0.0003130	0.0001200	0.0002100
Rainfed area (million ha)	0.0026815						
Irrigated area (million ha)	0.0026815	0.0028820	0.0001500	0.0005300	0.0003130	0.0001200	0.0002100
Irrigation water use (m^3/ha)[2]	20,562	23,058	27,218	13,185	7,289	11,517	6,662
Total irrigation use (MCM)	55.14	66.45	4.08	6.99	2.28	1.38	1.40
Yield (kg/ha)	2,080	10,900	11,962	8,553	8,571	4,523	8,676
Farm gate price ($/kg)	0.42105263	0.610	0.42	0.3	0.28	1.41	0.41
Gross revenue ($/ha)	876	6,649	5,024	2,822	2,400	6,377	3,557
Water related costs ($/$m^3$)	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Capital costs ($/$m^3$)	0.044	0.04	0.04	0.04	0.04	0.04	0.04
Maintenance ($/$m^3$)	0.0038	0.00	0.00	0.00	0.00	0.00	0.00
Operation - diesel ($/$m^3$)	0.017	0.02	0.02	0.02	0.02	0.02	0.02
diesel use (L/m^3 water)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cost of diesel ($/L)	0.094	0.09	0.09	0.09	0.09	0.09	0.09
Operation (Oil $/$m^3$)	0.002	0.00	0.00	0.00	0.00	0.00	0.00
Labor costs ($/ha)[3]	124	450	434	410	398	405	424
Other cost ($/ha)[4]	120	250.00	230	212	215	212	231
Irrigation water applied (m/ha)	2.06	2.31	2.72	1.32	0.73	1.15	0.67
water cost ($/ha)	1,339	1,532	1,804	873	482	761	440
Total costs ($/ha)	1,583	2,232	2,468	1,495	1,095	1,378	1,095
Net revenue ($/ha)	707-	4,417	2,556	1,327	1,305	4,999	2,462
Returns to water ($/$m^3$)	0.0	0.3	0.2	0.2	0.3	0.6	0.5

[1] Ag. S. Sources of Area and productivity are the Agricultural Year Kook – 2002 to 2006.
[2] Grw. S. P. Groundwater & Soil Conservation Project, Sauyen Hadramout December 2007.
[3] DRC المصادر: تقرير التنمية البشرية ، الإحصاء الزراعي لعام 2006، د. عبد العزيز -خبير الفاو.
[4] Other costs (manure, urea, chemical fertilizers, chemicals) ($/ha).

Table 4. Crop budgets input output analysis of the main cropping patterns in Wadi Hadramout 2003.

Cropping pattern	Dates	Alfafa	Banana	Onion	Tomato	Garlic	Potato
Area cultivated(million ha)[1]	0.01	0.0023830	0.0002100	0.0010200	0.0002410	0.0001130	0.0001790
Rainfed area (million ha)	0.003907						
Irrigated area (million ha)	0.00	0.0023830	0.0002100	0.0010200	0.0002410	0.0001130	0.0001790
Irrigation water use(m^3/ha)[2]	21,712	23,993	28,488	13,365	7,540	12,097	7,371
Total irrigation use (MCM)	84.83	57.17	5.98	13.63	1.82	1.37	1.32
Yield (kg/ha)	1,384	10,950	11,953	8,533	8,578	4,513	8,622
Farm gate price ($/kg)	0.34	0.58	0.53	0.3	0.38	1.3	0.35
Gross revenue ($/ha)	473	6,351	6,335	2,645	3,260	5,867	3,018
Water related costs ($/$m^3$)	0.066	0.067	0.07	0.07	0.07	0.07	0.07
Capital costs ($/$m^3$)	0.044	0.04	0.04	0.04	0.04	0.04	0.04
Maintenance ($/$m^3$)	0.0038	0.00	0.00	0.00	0.00	0.00	0.00
Operation - diesel ($/$m^3$)	0.017	0.02	0.02	0.02	0.02	0.02	0.02
diesel use (L/m^3 water)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cost of diesel ($/L)	0.095	0.09	0.09	0.09	0.09	0.09	0.09
Operation (Oil $/$m^3$)	0.002	0.00	0.00	0.00	0.00	0.00	0.00
Labor costs ($/ha)[3]	135	426	462	416	486	423	478
Other cost ($/ha)[4]	142	233.00	222	189	207	217	257
Irrigation water applied (m/ha)	2.17	2.40	2.85	1.34	0.75	1.21	0.74
water cost ($/ha)	1,429	1,612	1,913	898	506	812	495
Total costs ($/ha)	1,706	2,271	2,597	1,503	1,199	1,452	1,230
Net Revenue ($/ha)	1,232-	4,080	3,738	1,143	2,060	4,415	1,788
Returns to water ($/$m^3$)	0.02	0.26	0.22	0.20	0.43	0.48	0.41
Crop water requirement (m^3/ha)	14,379	15,580	18,145	8,970	5,027	8,174	4,725

[1] Ag. S. Sources of Area and productivity are the Agricultural Year Kook – 2002 to 2006.
[2] Grw. S. P. Groundwater & Soil Conservation Project, Sauyen Hadramout December 2007.
[3] DRC المصادر: تقرير التنمية البشرية ، الإحصاء الزراعي لعام 2006، د. عبد العزيز -خبير الفاو.
[4] Other costs (manure, urea, chemical fertilizers, chemicals) ($/ha).

Table 5. Crop budgets input output analysis of the main cropping patterns in Wadi Hadramout 2002.

Cropping pattern	Dates	Alfafa	Banana	Onion	Tomato	Garlic	Potato
Area cultivated (million ha)[1]	0.01	0.0023710	0.0001570	0.00054	0.00031	0.0001200	0.0002300
Rainfed area (million ha)	0.003856						
Irrigated area (million ha)[2]	0.00	0.0023710	0.0001570	0.00054	0.00031	0.0001200	0.0002300
Irrigation water use (m^3/ha)	18,693	21,811	25,948	12,638	7,339	11,770	6,662
Total irrigation use (MCM)	72.08	51.71	4.07	6.82	2.30	1.41	1.53
Yield (kg/ha)	1,350	10,973	11,600	8,526	8,559	4,526	8,683
Farm gate price ($/kg)	0.479	0.570	0.4	0.4	0.42	1.38	0.41
Gross revenue ($/ha)	646	6,255	4,640	3,410	3,595	6,246	3,560
Water related costs ($/$m^3$)	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Capital costs ($/$m^3$)	0.045	0.04	0.04	0.04	0.04	0.04	0.04
Maintenance ($/$m^3$)	0.0039	0.00	0.00	0.00	0.00	0.00	0.00
Operation - diesel ($/$m^3$)	0.018	0.02	0.02	0.02	0.02	0.02	0.02
diesel use (L/m^3 water)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cost of diesel ($/L)	0.096	0.10	0.10	0.10	0.10	0.10	0.10
Operation (Oil $/$m^3$)	0.002	0.00	0.00	0.00	0.00	0.00	0.00
Labor costs ($/ha)[3]	154	463	473	412	498	415	469
Other cost ($/ha)[4]	124	189.00	229	143	263	132	253
Irrigation water applied (m/ha)	1.87	2.18	2.59	1.26	0.73	1.18	0.67
water cost ($/ha)	1,243	1,481	1,761	858	498	798	452
Total costs ($/ha)	1,521	2,133	2,463	1,413	1,259	1,345	1,174
Net revenue ($/ha)	875-	4,122	2,177	1,998	2,336	4,900	2,386
Returns to water ($/$m^3$)	0.0	0.3	0.2	0.3	0.5	0.5	0.5

[1] Ag. S. Sources of Area and productivity are the Agricultural Year Kook – 2002 to 2006.
[2] Grw. S. P. Groundwater & Soil Conservation Project, Sauyen Hadramout December 2007.
[3] DRC المصادر: تقرير التنمية البشرية ، الإحصاء الزراعي لعام 2006، د. عبد العزيز -خبير الفاو.
[4] Other costs (manure, urea, chemical fertilizers, chemicals) ($/ha).

The Date Palm and Its Role in Reducing Soil Salinity and Global Warming

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Keywords: date palm, dates, *Phoenix dactylifera*, Iraq, global warming

Abstract

The date palm is a blessed tree that is known for its various benefits. It plays a big role in achieving environmental balance, as it grows in a harsh climatic environment and even in highly saline sand. Moreover, the date palm absorbs carbon dioxide to a significantly greater extent than other trees, due to its large size. It also on average stores more carbon than other trees of similar size. This potential makes the date palm an important tool in the fight to stave off global warming, which is mainly caused by carbon dioxide emissions. Consequently, this paper proposes a massive date palm tree planting exercise in the Arab world, in order to make it the world's future lungs that would transform carbon dioxide into oxygen and food, in a similar way to the Amazon's rainforest. The paper makes a number of recommendations, including the need for taking good care of this neglected tree, the provision of government incentives for growing it, and the inclusion of the planting of date palms as part of carbon offset projects which would be incorporated into an overall sustainable development agenda.

INTRODUCTION

Date palm (scientific name *Phoenix dactylifera*) plantations exist in several regions of the world, but they are particularly concentrated in the MENA region (Fig. 1). The date palm is one of the most prominent trees in the Arab world in general, and in Iraq in particular where it is considered to be the national tree that is distinguished globally. The date palm has played a very important role in the economic, social and religious aspects of life for generations because it was one of the most vital sources of food for date palm cultivators. Estimates indicated that in Iraq there were 30 million date palm trees in 1970. The scars of war and economic crises were among the most significant reasons for the deterioration of the date palm industry in Iraq. During the first Gulf War (1980-1988) and the second (1991), more than 20 million palm trees were completely destroyed in Iraq. It is estimated that there are 350-400 date types, including high quality dates. Notably, the date was considered to be Iraq's second largest national export after oil. According to a report by the United Nations Food and Agriculture Organization (FAO) Iraq was - before the embargo - the largest date producer, producing 550,000 tons per annum. This is equivalent to 80% of the world market and generated an annual revenue of nearly 50 million dollars for Iraq.

However, the situation has changed greatly; Iran, Egypt and Saudi Arabia exceeded Iraq in terms of exportation, to the extent that Iraq's export figures no longer appear officially at the international level. By contrast, Tunisia occupied first place in terms of world's date exports with an annual income equal to 47 million dollars followed by Pakistan with 23 million dollars and Iran with 21 million dollars. The FAO reports have confirmed that the United Arab Emirates has stepped up its date exports by a factor of four times between 1989 and 1993. However, dates exports rose even higher in Iran, from 10,000 tons in 1989 to 60,000 tons in 1993. In 1994, Iraq's date palm productivity was around 20-25 kg for one date palm, whereas, in the same year, date palm productivity reached 60-70 kg in Saudi Arabia (Al-Rawi, 1996).

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DISCUSSION

Reasons behind the Decline of the Date Palm

There has been a major decline in interest in date palms since the 1960s and this negligence had doubled by the 1980s. Several reasons have been attributed to this decline in palm tree cultivation. These include:

1. Urban sprawl.
2. Lack of government interest and lack of serious plans for date palm plantations.
3. Negligence of farmers and failure to use technology for date palm cultivation.
4. Shortage of water and high soil salinity.
5. Effects of war upon the agriculture sector and the economic blockade which accelerated the depletion process of date palms in Iraq. This depletion began with the first Gulf War and has continued until the present day, however only recently a change of the trend is observed and Iraq starts planting more date palms (United States Agency for International Development, 2005).

Characteristics of the Date Palm

The date palm is a unique type of palm tree. This is because it is deciduous and wind pollinated. There are nearly 500 types of date palms. The date palm is tall and can grow to a height of 24-30 meters. They can live for more than 200 years. Date palm leaves are beautiful and they normally do not fall off until the tree dies. Date palms can grow in hot arid regions, with temperatures ranging between 24 and 34°C. They adapt well to a harsh climatic environment, even with water scarcity and excessively high temperatures. However, it is sometimes argued that it would be best to grow date palms in areas which are characterized by long and hot summers (during which dates mature), with winter temperatures not less than -9°C. Such climates exist in the Arabian Peninsula, Iraq and the South-West region of Iran, where high quality dates mature perfectly while on the tree.

Moreover, date palms can grow in different types of soil, including dry, clay and sandy soils. The table below, which demonstrates the salt tolerance for different types of plants, shows that the date palm is best adapted to a high salt tolerance environment (i.e., 2000-500 ppm). Recent studies even indicate that the range is, in fact, 6000-7000 ppm (Barreveld, 1993). It should be noted however that, depending upon the environment and the type of soil, the productivity of the date palm decreases as the salt concentration increases.

It is commonly known that photosynthesis refers to the process that converts carbon dioxide into organic compounds such as sugars (i.e., glucose) using the energy from sunlight. Date palms absorb carbon dioxide (CO_2) and produce sugar, oxygen (O_2) and water (H_2O), similar to other plants, as shown in the following formula:

$$6\ CO_{2(g)} + 12\ H_2O_{(l)} + \underline{\text{photons}} \rightarrow \underline{C_6H_{12}O_{6(aq)}} + 6\ O_{2(g)} + 6\ H_2O_{(l)}$$

Based on this formula, 1.46 ton of CO_2 and 1.2 ton of water can produce 1 ton of sugar, 0.53 ton of oxygen and 0.6 ton of water. This translates to the net use of only 0.58 tons of water. The sugar produced forms a main source of food for humans and animals, both of which are the main polluters of the environment. Indeed, the amount of absorbed CO_2 depends on the size of the plants' green parts. Here, it is perhaps worth acknowledging that the date palm is a large tree with very dense leaves (the length of a leaf is 4-5 m), and each leaf has approximately 150 leaflets (each leaflet is around 30 centimetres in length and 2 centimetres in width). Moreover, given that the height of the date palm tree ranges from 15 to 25 metres, its absorption of CO_2 is significant. Figure 2 illustrates the parts of the date palm tree.

Moreover, carbon constitutes 50% of the dry wood, whilst water represents around 75% of the whole living plant. With regard to the date palm, water constitutes a maximum of 25%, whilst carbon makes around 60% (all of which has been absorbed from the

atmosphere). It should be noted that although the amount of captured carbon depends on the type and age of the tree, carbon is also stored in the trunk and roots. It is common knowledge that burning 1 ton of carbon produces 3.66 ton of CO_2 as per the following reaction:

$$C + O_2 = CO_2$$

In other words, the production of 1 ton of carbon requires around 3.66 ton of CO_2. Given that not only is the date palm a long living tree (with an age that might exceed 100 years), and is also a large tree with the biggest roots (with wood density of around 200-900 kg/m^3 (Khani, 2005)), comparatively significant amounts of CO_2 are absorbed and stored in the trunk and roots of the tree in a form of carbon. Assuming a height of 15 m and a diameter of 0.5 m, the mass of the trunk woods is approximately 1,472 kg. Thus, water constitutes: 1,472×25%=368 kg. The remaining solid part would represent 1,472–368=1,103.8 kg. The carbon mass would be around 1,103.8×50%=552 kg. This would lead to a total lifetime amount of carbon dioxide of around: 522×3.66=2020.3 kg of CO_2.

Another distinctive feature of the date palm is its productivity. It is estimated that during the first 5 to 8 years of its life, the yearly crop is around 8-10 kg. The total crop, up to 13 years of age, is around 60-80 kg. When the date palm is grown in a suitable agricultural and climatic environment, with proper care, its crop could reach 100 kg per year (Morton, 1987). About 79% of that weight is carbohydrate which is a form of carbon. This would mean that a mature date palm, in addition, to storing a large amount of carbon in its trunk and roots, it converts a large amount of CO_2 which is annually equivalent to about 290 kg, as well as producing food.

Date Palm: Environmental and Economic Implications

Like all plants, the date palm has a positive impact on the environment, not to mention its aesthetic advantage in decorating roads and improving the landscape. It provides shade and comfort to human life. According to recent studies, the date palm has the potential for improving the environment of the Arab world through the following:

1. Given the adaption features of the date palm, it has an important role in enhancing ecological balance and reducing desertification. The intensification in the growing of date palms near cities could work as a shield against dust storms.
2. Palm trees have a big potential in absorbing CO_2 from the atmosphere; something that is of high priority to a wide range of governmental and non-governmental organizations. Compared to other plants of a comparable size, the palm tree needs a minimal amount of water. In an earlier study (Sharif, 2008), it was demonstrated that one million mature date palm trees can absorb 2.0 million tons of CO_2. Based on photosynthesis calculations, we can suggest that since one date palm tree would lead to a reduction of CO_2 by 200 kg annually, growing a million trees would absorb 200,000 tons of CO_2 as well as the food value.
3. Besides its high CO_2 absorption potential, date palms have a high storage capability given its large size and long life.
4. Given that the annual crop of dates from a single date palm could exceeds 100 kg (Morton, 1987), growing date palms on a large scale could generate both high revenue and jobs.
5. Within date palm farms, there tend to be large spaces between palms. These could be used to grow different types of fruits such as grapes (which also have a high carbon content and nutritious value).

Iraq as the World's Future Lung

Considering the CO_2 emissions in England - for instance - it has been estimated that it produced 566.7 million tons of CO_2 in 2007. This makes the country among the highest CO_2 producers in the world (bearing in mind that the United States of America produce 5,877 million tons). Growing 100 million palm trees could absorb up to 200

million tons of CO_2 (i.e., the equivalent of 30% of England's total emissions). Growing this number of date palms would require 3,600 km^2 (i.e., around 1.5% of England's land area), assuming that one km^2 would be enough to grow 30,000 date palms. It should also be noted that not all of these date palms need to be grown on a single farm. However, given that the climatic environment of England is not suitable for growing date palms; other counties, with more suitable environments, such as Iraq or other MENA countries, could contribute in such an endeavor.

In this regard, it is proposed to intensify date palm cultivation in Iraq, which once had at least 30 million palm trees. Figure 3 shows that Iraq, among a number of other Arab countries, leads the world in terms of date crops. This would essentially make Iraq the world's future lungs that would enable it to inhale CO_2 and exhale oxygen, in a similar way to the Amazon rainforest, as well as producing high value and quality food. If the target of 30 million palm trees is achieved, it is possible that 6 million tons of CO_2 would be absorbed annually, not to mention approximately 60.0 million tons of carbon that would be stored in the trunk and roots, during the life time of the trees.

Moreover, it should be remembered that Iraqi dates are among the highest quality and rarest type in the world. This means that growing date palms in Iraq is likely to generate high economic returns, whilst providing employment opportunities. There is no doubt, given the magnitude of such an ambitious project, that Iraq would require regional and international support in terms of the planning, funding and scientific aspects. It is worth acknowledging here that, as a result of the support provided by the United States Agency for International Development (USAID), interest in growing date palms has been revived to the extent that 410,000 date palms have been planted per annum, in different parts of Iraq since 2003 (United States Agency for International Development, 2005).

Date Palms and Sustainable Development

Given the recent environmental concerns, there have been many calls for providing solutions that would ultimately lead to sustainable development. The most cited definition of sustainable development is perhaps the one provided by the World Commission on Environment and Development in 1987: "Development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (World Commission on Environment and Development, 1987). The world, as a whole, is trying to find a compromise between economic growth and preserving natural resources, whilst taking into account the economic, environmental and social aspects of sustainable development. One of the most sustainable ways of achieving this is land cultivation and the greening of the earth. In this regard, it is argued that the date palm has a strong potential for achieving sustainable development through providing shade, achieving an ecological balance, producing food and improving the air quality. Therefore, it is of strategic importance to intensify the growing of date palms. It is also worth mentioning here that 'carbon offset projects', which are gaining momentum around the world, could be used to encourage the cultivation of palm trees. Carbon offsetting, which refers to the idea of assuaging guilty consciences as a result of the production of greenhouse gases (including CO_2), offers a range of ways for individuals and businesses to participate in global warming solutions. The basic idea is to figure out the 'carbon footprint', which refers to the individual's contribution to the global warming problem, and then attempt to balance out this footprint through buying carbon offsets which could be used for a wide range of environmentally-friendly projects such as the development of renewable energy and tree planting projects. It is proposed here to place date palms at the top of the tree planting initiatives in Iraq (and the Arab world in general), as part of the carbon offsetting endeavours. This would help in realising the ambition of making Iraq the future lungs of the world by inhaling CO_2 and exhaling oxygen.

CONCLUSIONS

Date palms have a strong potential in addressing the problem of global warming, along with tackling air pollution and soil salinity problems. Plans must be put into place

in order to effectively utilize date palms and make them the future lungs of the world. Awareness of the benefits of date palms has to be raised among decision makers, investors and the general public. There is a need to organize conferences and seminars that explain the potentially important role of date palms. Moreover, funds need to be allocated to support research projects aimed at improving the cultivation of date palms. Last, but certainly not least, growing date palms needs to be incorporated into sustainable development initiatives and carbon offset projects.

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Table

Table 1. Salt tolerance for different types of plants.

Low salt tolerance	Medium salt tolerance	High salt tolerance
Pear almond	Pomegranate	Date palm
Apple apricot	Fig	
Orange peach	Olive	
Grapefruit strawberry	Grape	
Prune lemon	Cantaloupe	
Plum avocado		

Figures

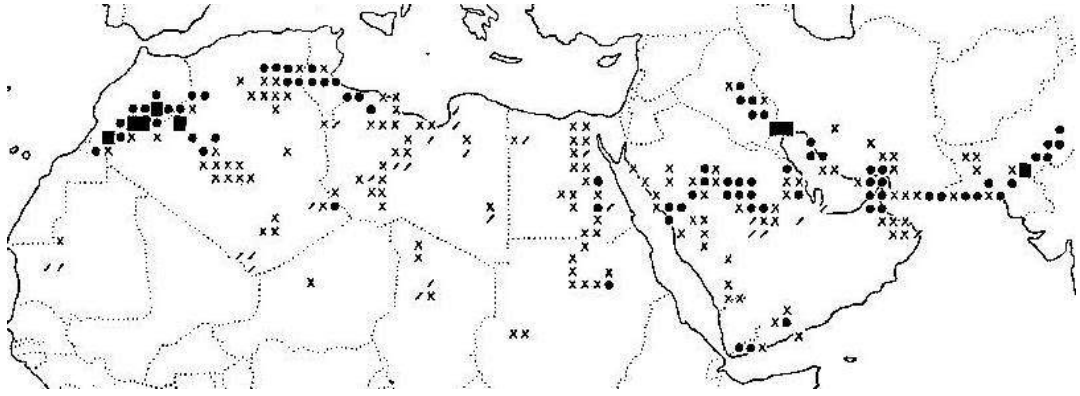

Fig. 1. Distribution of date palm plantations in the mid-1960s.

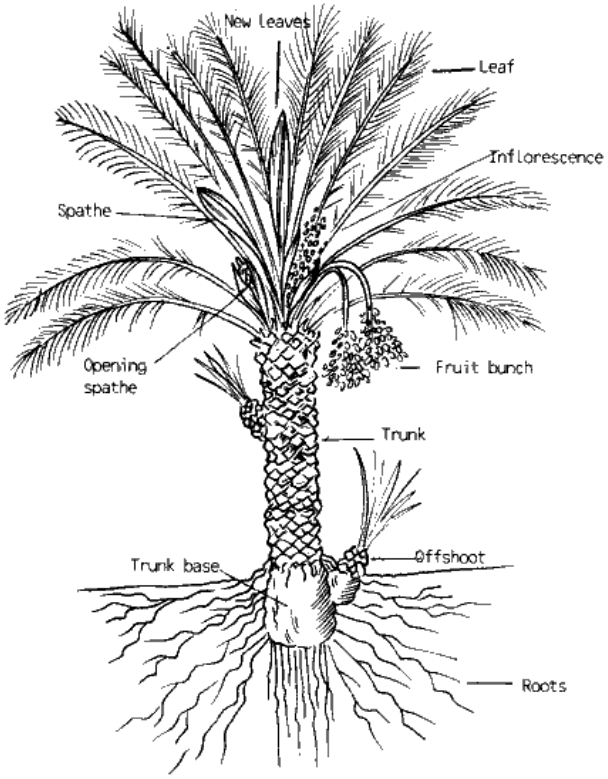

Fig. 2. Schematic illustration of date palm tree and its parts.

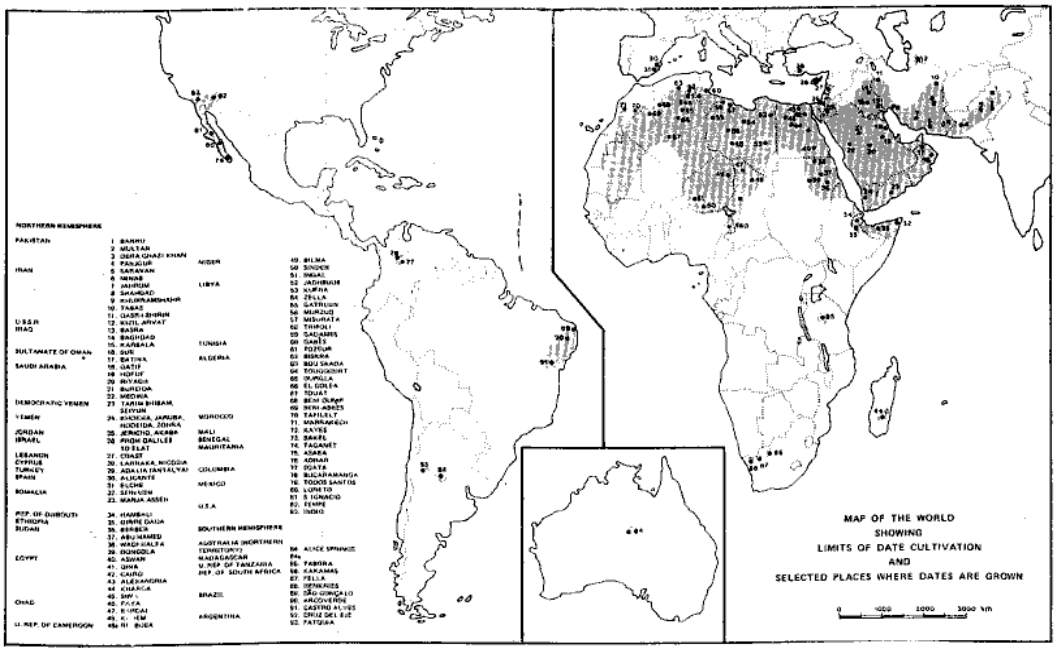

Fig. 3. International spread of date cultivation.

Performance and Field Management of Six Prominent Cultivars of Date Palm (*Phoenix dactylifera*) in Extreme Arid Regions of Thar Desert, India

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Keywords: date palm, Indian Thar Desert, doka and pind stage, fruit characteristics

Abstract

Ten female cultivars of datepalm were introduced in 1996 at the Chandan research centre located at 27°01'N and 71°01'E in Jaisalmer district of Western Rajasthan, India. Twelve offshoots of six prominent cultivars, each having average weight of 10-12 kg were further transplanted after careful separation from mother plants in the year 2000. It is revealed that transplanting of offshoots in the month of September is more beneficial with higher survival; 90% as against 65% in the offshoots transplanted in the month of March-April. First flowering was observed in 'Halawi' and 'Samran' in the second year of field planting whereas other cultivars flowered in the fourth and fifth year except 'Migraf', which flowered in the sixth year. Minimum thermal degree days were required for 'Khadravi'; 2213°C for khalal stage to 3125°C for tamar (dang) stage, whereas maximum was recorded in 'Medjool'; 2640°C for khalal stage to 3440°C for tamar stage during 2009. Physical characteristics of fruits of six prominent cultivars revealed a maximum fruit length of 39.5 mm in 'Medjool' and minimum 25.1 mm in 'Khadrawi'. Similarly, maximum pulp thickness of 16.3 mm was recorded in 'Medjool' and least 7.4 mm in 'Dayani' at khalal stage. The single fruit weight 16.1 g was also highest in 'Medjool' and lowest 3.8 g in 'Khadrawi'. Significant reduction in pulp and net weight of single fruit was recorded in all the cultivars, which was up to 30-35% in 'Halawi'. Maximum number of bunch 15 were recorded in 'Khalas' followed by 14 in 'Halawi'. Maximum yield of 125 kg per plant at tamar stage was recorded in 'Khalas' followed by 'Halawi', 92 kg and closely followed by 'Medjool', 89 kg during the year 2009. The prevailing conducive climatic conditions along with the inception of a canal system and ground water in the desert has opened the potential of commercial cultivation of date palm in the Indian Thar desert in millions of hectares in the future ahead.

INTRODUCTION

Date palm is the most potential plant among different crops in the Thar desert of India due to the conducive climatic conditions in the region and its high prospect and promise for obtaining high productivity and sustaining livelihood security. In recent times, the inception of the canal irrigation system in the most western districts in the Thar desert has further enhanced the possibility of harnessing the full potentiality of the date palm crop. The introduction trials on several cultivars of datepalm at the Chandan research centre of the Central Arid Zone Research Institute, India, have generated valuable information on fruit development stages of datepalm. It is only the Jaisalmer district in India where the long dry period of 180-185 days with cumulative heat summation above 3000°C are available, which helps to produce tree ripened soft dates every year (Chandra et al., 1992; Chundawat, 1990; Mertia and Vashishta, 1985; Mertia

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et al., 1995; Pareek and Sodagar, 1986; Singh et al., 2000). In the present study, six prominent cultivars of date palm have been evaluated through several field trials.

MATERIALS AND METHODS

The Geographical Setting and Climate

The experiment has been laid out in the year 2000 at the research centre Chandann (27°01'N and 71°01'E) of the Central Arid Zone Research Institute, Regional Research Station, Jaisalmer. It is situated at the heart of the Thar desert in the most western part of India. Agro-climatically, Jaisalmer is characterized by low and erratic rainfall, extreme temperatures, and high evapotranspiration rate. Monsoon sets in the region during the second week of July and starts to recede by the end of August. Long term average annual rainfall of the study region is 180 mm, 80% of which occurs during the monsoon season.

Soil Site Condition

Chandan farm is situated on undulating aeolian plains with stabilized and denuded sand dunes. The soils are deep, mixed hyperthermic, calcareous family of Typic Torripsamnents (Table 1). The soils are low in organic carbon (0.12%), low in available N content (97 kg $KMnO_4$-N ha^{-1}), low in P content (7 kg P ha^{-1}), and medium in K content (130 kg K ha^{-1}) (Table 1).

Experimental Setting

Twelve offshoots of six prominent cultivars were planted in the field after separation from mother plants in the year 2000 (Mertia et al., 1995). Observations on vegetative and reproductive attributes were recorded annually. Four trees of each cultivar were used as a replicate in randomized block design. They were evaluated in respect of physical characters of fruits at doka (khalal) and pind (tamar) stage in the fifth year of field planting when all the cultivars produced a sufficient quantity of fruits.

RESULTS AND DISCUSSION

It was revealed during separation of offshoots from mother plants that 10-12 kg offshoot weight led to better establishment over others. Similarly, transplanting in the month of September showed higher survival (90%) as against 65% when transplanted in the months of March-April.

Physical characteristics of fruits of six cultivars at doka (khalal) stage revealed amaximum length of fruit in 'Medzool' (39.5 mm) followed by 'Shamran' (34.0 mm) and lowest in 'Khadrawi' (25.1 mm). Maximum pulp thickness was also recorded in 'Medzool' (16.3 mm) followed by 'Burhee' (11.8 mm) and minimum in 'Khadrawi' (6.6 mm). Maximum fruit weight was recorded in 'Medzool' (16.1 g) and minimum in 'Khadrawi' (3.8 g) (Table 2).

Similarly the data on fruit characteristics for above said six cultivars at pind (tamar) stage indicated drastic reduction in both the length and weight of fruits in all cultivars (Table 3). The cultivar 'Burhee' did not attain this stage and was consumed as fresh date.

The physico-chemical composition of fruit pulp at tamar stage revealed that the maximum total sugar content was in 'Umshok' (70.10%) followed by 'Dayani' (61.44%) and minimum in 'Khadrawi' (54.13%). The maximum amount of thiamine and riboflavin was also recorded in 'Medzool' (0.030 and 0.027%, respectively) (Table 4).

CONCLUSIONS

The cultivars 'Medzool' and 'Dayani' are suitable for dry dates but good quality soft dates are also formed. The early appearance of spathe in the last week of January to the first week of February and due to dry periods of 180-185 days lead to formation of soft dates on tree. Attaining the full stage of soft dates on the tree results in maximum loss of weight 30-35%, which may be avoided by harvesting fruits a little early at the

beginning of the tamar stage and fruits ripen within weeks time without much loss of weight and can be sold in the market.

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Tables

Table 1. Physio-chemical properties of the soils of the experimental site.

Mixed, hyperthermic, calcareous family of Typic Torripsamments									
Horizon	Depth (m)	Particle size distribution			$CaCO_3$ (%)	pH	EC (dS m^{-1})	Organic carbon (%)	CEC (Cmol (+p)/kg)
		Sand (%)	Clay (%)	Texture					
Ap	0-20	77.4	6.1	ls	5.4	8.7	0.8	0.12	3.5
C1	20-55	83.2	6.8	ls	4.0	8.6	0.5	0.08	2.8
C2	55-108	86.1	6.5	ls	5.2	8.6	0.3	0.06	2.4
C3	108-137	86.7	6.7	ls	6.2	8.7	0.4	0.06	2.4

ls = loamy sand

Table 2. Physical characteristics of date palm fruits at doka (khalal) stage of six cultivars grown at Chandan farm, Jaisalmer.

Cultivar	Fruit dimension (mm)		Stone dimension (mm)		Pulp thickness (mm)	Fruit weight (g)		
	Length	Dia	Length	Dia		Pulp	Stone	Total
Medzool	39.5	25.9	20.3	9.6	16.3	15.0	1.1	16.1
Shamran	32.6	21.2	21.9	9.7	11.5	8.5	1.2	9.7
Barhee	34.0	21.9	24.9	10.1	11.8	8.4	1.4	9.8
Dayani	31.6	16.9	23.1	9.5	7.4	4.2	1.3	5.5
Khadrawy	25.1	15.2	19.4	8.6	6.6	2.9	0.9	3.8
Umshock	29.9	19.2	22.5	9.7	9.5	5.8	1.3	7.1
CD (p=0.05)	2.8	1.2	1.9	0.9	1.2	1.2	0.3	1.0

Table 3. Physical characteristics of date palm fruits at pind (tamar) stage of six cultivars grown at Chandan farm, Jaisalmer.

Cultivar	Fruit dimension (mm)		Stone dimension (mm)		Pulp thickness	Fruit weight (g)		
	Length	Dia	Length	Dia	(mm)	Pulp	Stone	Total
Medzool	32.3	21.4	17.4	8.3	13.1	9.4	0.8	10.2
Shamran	30.1	19.2	18.9	7.8	11.4	6.8	0.8	7.6
Barhee	-	-	-	-	-	-	-	-
Dayani	28.6	13.9	18.6	8.4	5.5	2.6	1.0	3.6
Khadrawy	22.8	14.2	16.6	7.1	7.1	2.9	0.6	3.4
Umshock	30.1	19.9	21.9	8.5	11.4	4.5	0.9	5.7
CD (p=0.05)	2.6	1.6	1.7	0.9	1.4	0.6	0.1	0.7

Table 4. Physico-chemical composition of date palm fruits at pind (tamar) stage of six cultivars grown at Chandan farm, Jaisalmer.

Cultivar	Total sugar (%)	Thiamine (%)	Riboflavin (%)	Nicotinic acid (%)	Ascorbic acid (%)
Medzool	56.63	0.030	0.027	0.30	-
Shamran	59.26	0.013	0.019	0.31	-
Dayani	61.44	0.026	0.014	0.16	-
Khadrawy	54.13	0.009	0.023	0.19	-
Umshock	70.10	0.004	0.031	0.29	-

Evaluation and Selection of Some Seedling Date Palm Males Grown in Fayoum Governorate, Egypt

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Keywords: date palm, evaluation, spathe characteristics, strand characteristics, pollen grains

Abstract

This study was conducted in Tamiya district, Fayoum Governorate, Egypt in three successive seasons of 2005, 2006 and 2007. Fifty seedling palm males were chosen and evaluated in order to select the suitable and most promising males of them to be used in pollinating female date palms. The results showed that there are five palm males (No. 2, 10, 29, 40 and 46) that were characterized as highly potent and that recorded the highest grades for the studied characters. These characters involved: weight and viability of pollen grains, spathes number, flowers number, start and duration of spathe burst. Accordingly, those selected palm males could be used as pollinators for date palm females and considered as recommended under the conditions of this study.

INTRODUCTION

In most date palm growing countries including Egypt, date growers are accustomed to use pollens from seedling males in pollinating female palms where readily available. These seedling males are highly variable, in the sense that they differ greatly in their growth, vigour, flowering time, spathe characteristics and pollen quality (Nixon, 1959; El-Sabrout, 1979; Shaheen et al., 1989; Gasim, 1993 and Dawoud, 2001). As a result, there is a direct effect on yield and fruit quality of the palms. Many investigators cleared that different pollen sources have a direct effect on fruit-setting, fruit qualities and the time of fruit ripening on several date cultivars (El-Hammady et al., 1977; Dawould, 2001; Moustafa, 2001). They also added that this effect varies according to the male parent used in pollinating female palm trees.

Fayoum Governorate, is one of the main areas of date production in Egypt, especially semi-dry date cultivars. There are large variations in fruit characteristics. This implies that the selection of male palm trees which is used as appropriate pollinators is very important to ensure a high yield with the best fruit qualities.

The main goal of this study is to evaluate different male palm trees, then select the suitable and most promising males of them to be used in pollinating female date palms.

MATERIALS AND METHODS

This study was conducted during three successive seasons of 2005, 2006 and 2007 on fifty seedling palm males. Palms were about 20 years old and grown in a clay loam soil at Tamiya district, Fayoum Governorate, Egypt. No special treatments were given to the palms more than the normal field cultural practices. All experimental palm males were chosen and marked from 1 to 50 in order to be evaluated in this study.

In each season, the dates of the beginning and ending of spathe burst for each male were recorded and spathe burst duration as well as spathes number were calculated. Morphological spathe characteristics for each male such as weight, length and width were recorded, then the averages were calculated. Also, the characteristics of strand were recorded and calculated where involved: strands number per inflorescence, strand length

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and flowers number per strand for each palm male.

For pollen grains characteristics, the strands of each spathe were cut off and spread in a thin layer on paper sheets for 3-4 days till they became dry. Then, the pollen grains were separated from the floral parts using fine sieves (40 mesh). The pollen grains of each experimental palm male were collected and their weight was determined. As soon as pollen grains were collected, pollen viability was tested with acetocarmen-staining. One drop of 1% acetocarmen was placed on pollen and microscopically examined. Colorless or unstained pollen grains were considered non viable. Ten counts at different fields were examined and readings were recorded to determine the percentage of pollen viability according to Hamdy (1982) and El-Salhy et al. (1997).

As for the selection of promising males, the 50 chosen seedling palm males were evaluated according to some characteristics suggested by Nixon (1959). These characteristics included: the amount and viability of pollen grains produced form the male, number of flowers per strand, number of spathes and flowering time. Different degrees were given to these characters as follows: 5 degrees for spathes number, 10 degrees for flowers number, 3 degrees for beginning of spathe burst, 2 degrees for duration of spathe burst, 30 degrees for weight of pollen grains and 50 degrees for viability of pollen grains. Then the score of each evaluated male palm was calculated on the basis of 100 degrees for the sum of degrees of the previous characteristics. The grade of any character for each palm male and the grade of any palm male for each characteristic were calculated as follows:

$$Grade\ character = \frac{L_2 - L_1}{Degrees\ which\ gave\ for\ character}$$

$$Grade\ male = \frac{M - L_1}{Grade\ character}$$

Where L_2 = maximum value of character for all tested palm males; L_1 = minimum value of character for all tested palm males; M = male value for character.

RESULTS AND DISCUSSION

Evaluation of Date Palm Males

1. Flowering. Spathe burst (date and duration) of 50 chosen date palm males are presented in Table 1. The obtained results on spathe burst times, which was calculated as the number of days after February 19 to burst the first spathe on each palm male show that the commencement of spathe burst varies to some extent according to males and seasons. It is clear from these results that the spathe burst of males No. 3 and 8 started about 19 days earlier (February 20) than those of males No. 6 and 14 (March 11). The spathe burst times of other palm males were in between. These results confirm the findings of Nasr et al. (1986), Desouky et al. (1993), Gasim (1993) and Dawoud (2001), who reported that date palm males differed greatly in flowering dates. Moreover, El-Amer et al. (1993) found that the palm males grown under Egyptian conditions usually commence flowering from February 3 till the first ten days of March.

Concerning the duration of spathe burst for each palm male which was calculated as the number of days between the burst date of the first spathe and those of the last one on each palm male, the results cleared that there was great variation according to the male type, where the longest was 44 days for male No. 13 and the shortest was 26 days for male No. 4. El-Amer et al. (1993) found that the flowering duration of palm males ranged between 31 to 43 days.

2. Spathes Number. Data in Table 1 show that the average number of spathes produced per male varied greatly according to the evaluated males. Increasing the spathes number produced per male decreased the risks in the amount of harvested pollen grains with loss or defect of some spathes. It is clear from these data that male No. 10 had the highest average number of spathes (33.33), while male No. 35 had the lowest number (9.33). These findings are supported with what was reported by El-Amer et al. (1993) and Abd

El-Rawy (2001), who found that the spathes number produced per palm male ranged from 22 to 41 and from 16 to 31 spathes, respectively according to the evaluated male.

3. Morphological Characteristics of the Spathe. The results obtained from the evaluation of male palms showed great variability in the morphological characteristics of the spathes of various males. These results (Table 2) are summarized as follows:

Spathe Weight. It was noticed that there are clear differences in the average spathe weight of the evaluated males. The highest weight was obtained for male No. 2 (2.68 kg) and the lowest one was found for male No. 47 (1.06 kg). These results are in agreement with the findings of El-Amer et al. (1993) and Abd El-Rawy (2001), who found that the average spathe weight in the different palm males varied from 110 to 1619 g and ranged from 1.7 to 2.8 kg, respectively.

Spathe Length. The average spathe length of the studied palm males ranged between 47.72 (male No. 40) and 90.07 cm (male No. 23). The results are in agreement with the findings of Nasr et al. (1986) who found that the spathe length of different date palm males ranged between 25 and 119 cm. Moreover, Abd El-Rawy (2001) pointed out that spathe length values were obtained of 79, 73, 87 and 85 cm of 'Khalaf', 'El-Galaly', 'El-Iraqui' and 'El-Bitary' palm males, respectively.

Spathe Width. There is a clear difference in the average spathe width among the studied palm males. The highest value was recorded for male No. 44 (19.51 cm) and the lowest value was recorded for male No. 26 (11.33 cm). Concerning this, Abd El-Rawy (2001) found that the average spathe width of different males ranged from 19.9 to 23.1 cm. Nixon (1959) stated that no two seedling palm are alike in the morphological characteristics of the spathes.

4. Characteristics of the Strand. Results pertaining to various characteristics of strands in the studied date palm males are shown in Table 3. These results are summarized as follows:

Strands Number/Inflorescence. It was found that the average number of strands of each inflorescence varied greatly among males. The highest number was recorded for male No. 39 (410.00 strands), while the lowest number was recorded for male No. 45 (114.33 strands). Differences in strands number per inflorescence of date palm males were reported by Nasr et al. (1986) who found that the strands number per inflorescence in different palm males varied from 23 to 420 strands. Moreover, Abd El-Rawy (2001) reported that the number of strands per inflorescence varied from 245 to 274 in different palm males.

Strand Length. The average length of strands inside the spathe clearly differed among different tested males. The longest strand was found for male No. 4 (34.30 cm) and the shortest strand was found for male No. 12 (12.67 cm). These results are supported by the findings of Nasr et al. (1986) and Abd El-Rawy (2001), who found that the average length of strands in different date palm pollinators was ranged between 5.20 and 38.10 cm and from 26.0 to 39.5 cm, respectively.

Flowers Number/Strand. There is a clear differences in flowers number per strand between the males where the highest average number of flowers was recorded for male No. 40 (104.00 flowers/strand) and the lowest one was that of male No. 6 (40.33 flowers/strand). These results are in line with those obtained by Nasr et al. (1986) and Abd El-Rawy (2001), who found that the number of flowers per strand in the different palm males ranged between 13.6 and 92.8 and from 78 to 109, respectively.

5. Characteristics of Pollen Grains. Results in Table 4 show the characteristics of pollen grains for 50 chosen palm males. These results (weight and viability of pollen grains) are summarized as follows:

Pollen Grains Weight. Results indicated that there was great difference among males in the average weight of pollen grains produced per palm male. The highest average weight of pollen grains was obtained for male No. 40 (1568.81 g) and the lowest weight was that of male No. 36 (24.26 g). El-Amer et al. (1993) reported that the amount of pollen grains produced per spathe of different males varied from 0.26 to 42.95 g, while Dawoud (2001) found that the pollen weight of spathes ranged between 25.30 and 83.10 g according to

the male type.

Pollen Grains Viability. There are great differences also among males in pollen grains viability. The highest percentage of pollen grains viability was obtained for male No. 3 (95.64%) while the lowest one was found for male No. 13 (67.59%). Concerning this, Nasr et al. (1986) found that the pollen grains viability of palm males ranged form 44.6 to 100.0% using the acetocarmin method and from 6.0 to 93.0% using the germination method. Moreover, Gasim (1993) reported that palm males varied in viability of pollen grains. Abd El-Rawy (2001) found that the pollen grains viability of four date pollinators varied between 82.3 and 92.0% according to the evaluated male.

Selection of Date Palm Males

The basis of selection of date palm males depends on some important characteristics as suggested by Nixon (1959) and Nasr et al. (1986). These characteristics included: flowering (start and duration of spathe burst), spathes number/palm, flowers number/strand and pollen grains (weight and viability).

Tables 5 and 6 show total scores estimated for the 50 chosen palm males. Those scores are the sum of the degrees which were given for each character from the previous characteristics. It is evident that 5 males are found to be higher in the total scores than the other males. They were numbers 2, 10, 29, 40 and 46 which had total scores of 80, 82, 80, 82 and 84%, respectively.

Concerning the start of spathe burst (Table 1), males numbers 2 and 10 had early spathe burst (at the last third of February), while the males numbers 29 and 40 had a medium of spathe burst (at the first 3 days of March). The male number 46 was the latest in spathe burst (at the first ten days of March). From a practical point of view, this could be useful, because it extends the duration of pollen grains harvesting. Moreover, the selected 5 males were close to each other in the characteristic of spathe burst duration (31 to 38 days).

As for the spathes number/palm (Table 1), flowers number/strand (Table 3) and pollen grains (Table 4), data show that the selected five males (No. 2, 10, 29, 40 and 46) had greater spathes number/palm (>20), a higher flowers number/strand (67.67 to 104.00 flowers), yielded higher amounts of pollen grains (1350.41 to 1568.81 g/palm) and had a high percentage of pollen grains viability (29.26 to 94.75%). In this regard, Nixon (1959) suggested that the basis of selection of male palms was the amount of pollen grains produced and the flowers should contain abundant pollen as well as the number of flowers/strand.

From the foregoing results, it can be concluded that the date palm males numbers 2, 10, 29, 40 and 46 were characterized as highly potent and recorded the highest scores as compared with other males. Accordingly, those selected palm males may be considered superior males and could be used as a pollinators for palm females under the conditions of this study.

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Tables

Table 1. Start and duration of spathe burst and spathes number of date palm males.

Start of spathe burst*				Duration of spathe burst				Spathes No.			
Male No.	Mean**	Male No.	Mean**	Male No.	Mean**	Male No.	Mean**	Male No.	Mean**	Male No.	Mean**
1	7	26	9	1	42	26	34	1	12.67	26	15.33
2	3	27	10	2	31	27	42	2	26.33	27	21.67
3	1	28	12	3	31	28	36	3	24.00	28	21.00
4	8	29	10	4	26	29	38	4	18.33	29	24.67
5	8	30	12	5	34	30	40	5	17.67	30	19.33
6	20	31	10	6	32	31	43	6	17.67	31	17.33
7	12	32	12	7	36	32	39	7	15.67	32	20.67
8	1	33	12	8	36	33	33	8	20.33	33	15.33
9	9	34	9	9	35	34	33	9	15.00	34	17.00
10	2	35	14	10	34	35	41	10	33.33	35	9.33
11	9	36	11	11	41	36	33	11	12.33	36	14.00
12	10	37	13	12	40	37	36	12	18.00	37	10.33
13	14	38	11	13	44	38	43	13	20.00	38	24.00
14	20	39	11	14	34	39	40	14	17.33	39	14.00
15	9	40	11	15	41	40	38	15	19.00	40	21.33
16	19	41	12	16	35	41	37	16	16.67	41	14.67
17	9	42	9	17	35	42	37	17	13.67	42	14.67
18	8	43	10	18	37	43	36	18	15.00	43	23.00
19	10	44	16	19	33	44	35	19	19.33	44	16.00
20	9	45	14	20	39	45	40	20	19.67	45	21.67
21	9	46	16	21	40	46	38	21	10.33	46	25.33
22	9	47	16	22	41	47	36	22	24.67	47	13.00
23	9	48	14	23	33	48	36	23	12.67	48	17.00
24	10	49	14	24	34	49	34	24	10.67	49	16.67
25	7	50	13	25	36	50	36	25	16.00	50	17.00

* No. of days after February 19 to burst the first spathe.
** Mean of seasons, 2005, 2006 and 2007.

Table 2. Morphological spathe characteristics of date palm males.

Spathe weight (kg)				Spathe length (cm)				Spathe width (cm)			
Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*
1	1.80	26	1.34	1	55.95	26	63.33	1	13.39	26	11.33
2	2.68	27	2.07	2	60.48	27	61.60	2	16.72	27	15.55
3	1.24	28	1.77	3	74.58	28	57.45	3	13.75	28	13.07
4	2.37	29	2.36	4	79.26	29	71.16	4	16.00	29	18.88
5	1.37	30	1.25	5	40.62	30	51.17	5	14.55	30	13.91
6	2.16	31	2.27	6	71.65	31	63.97	6	13.90	31	15.77
7	1.23	32	1.73	7	55.29	32	65.05	7	12.55	32	13.43
8	2.21	33	1.37	8	56.84	33	67.83	8	17.55	33	12.25
9	2.41	34	1.92	9	84.35	34	70.33	9	13.46	34	14.47
10	2.26	35	2.55	10	58.68	35	59.99	10	16.89	35	18.07
11	1.56	36	1.59	11	71.37	36	59.02	11	12.92	36	13.67
12	2.37	37	1.72	12	67.98	37	59.55	12	15.87	37	14.33
13	1.50	38	1.73	13	54.22	38	53.44	13	13.78	38	14.86
14	1.66	39	2.28	14	57.52	39	60.99	14	13.22	39	16.98
15	1.73	40	1.92	15	53.11	40	47.72	15	12.44	40	19.05
16	1.40	41	1.46	16	63.95	41	63.53	16	12.78	41	13.30
17	2.01	42	2.39	17	63.95	42	67.78	17	13.20	42	15.70
18	1.45	43	2.09	18	51.33	43	67.40	18	14.00	43	16.27
19	1.86	44	2.19	19	61.11	44	58.28	19	15.11	44	19.51
20	1.90	45	1.32	20	69.78	45	40.26	20	13.06	45	14.01
21	1.13	46	2.23	21	64.75	46	70.35	21	11.50	46	16.97
22	1.42	47	1.06	22	69.99	47	51.54	22	12.45	47	11.46
23	1.60	48	2.19	23	90.07	48	69.22	23	12.68	48	17.89
24	1.47	49	1.47	24	70.42	49	67.39	24	12.16	49	13.00
25	2.15	50	1.99	25	74.35	50	75.06	25	14.81	50	14.39

* Mean of seasons 2005, 2006 and 2007.

Table 3. Strand characteristics of date palm males.

Strands number/ inflorescence				Strand length (cm)				Flowers number/ strand			
Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*
1	239.11	26	231.24	1	18.45	26	21.38	1	61.67	26	60.00
2	321.50	27	186.59	2	22.30	27	20.17	2	67.67	27	62.00
3	190.61	28	191.19	3	30.79	28	18.67	3	53.67	28	80.33
4	219.01	29	283.63	4	34.30	29	26.42	4	61.33	29	84.67
5	205.86	30	171.64	5	13.59	30	22.74	5	56.00	30	59.00
6	213.66	31	289.40	6	28.69	31	20.66	6	40.33	31	86.00
7	182.30	32	229.67	7	20.14	32	22.27	7	66.67	32	67.00
8	278.65	33	207.89	8	18.47	33	27.44	8	68.33	33	75.67
9	412.58	34	409.55	9	27.47	34	18.54	9	76.33	34	55.33
10	249.48	35	227.22	10	24.40	35	18.64	10	86.67	35	72.67
11	258.06	36	313.94	11	14.51	36	17.32	11	81.33	36	68.33
12	303.18	37	286.58	12	12.67	37	14.78	12	71.67	37	63.00
13	324.27	38	229.38	13	20.27	38	13.31	13	81.67	38	81.33
14	279.97	39	410.00	14	21.03	39	22.82	14	89.67	39	61.00
15	181.40	40	238.70	15	19.33	40	17.14	15	75.67	40	104.00
16	197.39	41	277.10	16	31.81	41	17.60	16	67.33	41	69.00
17	373.12	42	368.89	17	20.60	42	18.96	17	79.33	42	81.00
18	230.91	43	353.27	18	22.40	43	33.40	18	85.33	43	91.00
19	169.42	44	257.68	19	23.56	44	21.52	19	69.33	44	96.33
20	347.54	45	114.33	20	27.63	45	28.17	20	83.33	45	73.67
21	312.23	46	299.51	21	19.83	46	19.03	21	43.67	46	77.33
22	191.89	47	282.74	22	27.88	47	17.27	22	72.67	47	54.33
23	204.84	48	223.64	23	23.85	48	23.10	23	81.00	48	89.33
24	204.26	49	258.99	24	20.56	49	23.20	24	53.33	49	85.67
25	211.62	50	262.76	25	21.84	50	19.79	25	86.33	50	70.33

* Mean of seasons, 2005, 2006 and 2007.

Table 4. Pollen grains characteristics of date palm males.

Pollen grains weight (gm)				Pollen grains viability (%)			
Male No.	Mean*	Male No.	Mean*	Male No.	Mean*	Male No.	Mean*
1	105.80	26	424.12	1	91.34	26	89.72
2	1350.41	27	499.51	2	94.75	27	91.25
3	566.99	28	46.09	3	95.64	28	88.32
4	420.02	29	1454.09	4	94.56	29	91.79
5	593.87	30	181.81	5	93.18	30	90.94
6	231.08	31	353.92	6	88.49	31	89.52
7	471.25	32	714.02	7	86.88	32	88.30
8	884.88	33	51.73	8	93.40	33	74.72
9	382.85	34	726.30	9	93.22	34	94.09
10	1487.08	35	35.79	10	92.00	35	84.88
11	32.46	36	24.26	11	87.25	36	93.01
12	924.89	37	406.85	12	93.14	37	79.89
13	318.03	38	1180.72	13	67.59	38	90.56
14	48.18	39	31.06	14	92.48	39	90.59
15	66.05	40	1568.81	15	93.31	40	89.26
16	380.16	41	375.30	16	88.50	41	89.61
17	25.95	42	80.37	17	91.27	42	90.68
18	447.01	43	710.22	18	93.59	43	79.48
19	434.31	44	617.18	19	89.24	44	82.77
20	63.64	45	759.89	20	78.40	45	89.86
21	404.82	46	1530.14	21	89.95	46	93.36
22	478.41	47	225.58	22	94.55	47	90.40
23	105.53	48	731.34	23	89.47	48	90.95
24	30.90	49	209.61	24	88.59	49	90.09
25	625.44	50	293.09	25	91.51	50	86.89

* Mean of seasons, 2005, 2006 and 2007.

Table 5. Total scores estimated for the fifty chosen palm males (Mean of seasons, 2005, 2006 and 2007).

Male No.	No. of spathes/palm (5)	No. of flowers/strand (10)	Spathe burst		Pollen grains		Total score (100)
			Beginning (days) (3)	Duration (days) (2)	Weight (g) (30)	Viability (%) (50)	
1	1	3	1	2	2	40	49
2	4	4	0	1	26	45	80*
3	3	2	0	1	11	47	64
4	2	3	1	0	8	45	59
5	2	3	1	1	11	43	61
6	2	0	3	1	4	35	45
7	1	4	2	1	9	32	49
8	2	4	0	1	17	43	67
9	1	6	1	1	7	43	59
10	5	7	0	1	28	41	82*
11	1	6	1	2	0	33	43
12	2	5	1	2	17	43	70
13	2	7	2	2	6	0	19
14	2	8	3	1	0	41	55
15	2	6	1	2	1	43	55
16	2	4	3	1	7	35	52
17	1	6	1	1	0	39	48
18	1	7	1	1	8	43	61
19	2	5	1	1	8	36	53
20	2	7	1	1	1	18	30
21	0	1	1	2	7	37	48
22	3	5	1	2	9	45	65
23	1	6	1	1	2	36	47
24	0	2	1	1	0	35	39
25	2	7	1	1	12	40	63

* Selected palm males.

Table 6. Total scores estimated for the fifty chosen palm males (Mean of seasons, 2005, 2006 and 2007).

Male No.	No. of spathes/palm (5)	No. of flowers/strand (10)	Spathe burst		Pollen grains		Total score (100)
			Beginning (days) (3)	Duration (days) (2)	Weight (g) (30)	Viability (%) (50)	
26	1	3	1	1	8	37	51
27	3	3	1	2	9	39	57
28	2	6	2	1	0	35	46
29	3	7	1	1	28	40	80*
30	2	3	2	2	3	39	51
31	2	7	1	2	6	37	55
32	2	4	2	1	13	35	57
33	1	6	2	1	0	12	22
37	2	2	1	1	14	44	64
35	0	5	2	2	0	29	38
36	1	4	2	1	0	42	50
37	0	4	2	1	7	21	35
38	3	6	2	2	22	38	73
39	1	3	2	2	0	38	46
40	3	10	2	1	30	36	82*
41	1	4	2	1	7	37	52
42	1	6	1	1	1	38	48
43	3	8	1	1	13	20	46
44	1	9	2	1	12	25	50
45	3	5	2	2	14	37	63
46	3	6	2	1	29	43	84*
47	1	2	2	1	4	38	48
48	2	8	2	1	14	39	66
49	2	7	2	1	4	38	54
50	2	5	2	1	5	32	47

* Selected palm males.

Cultivation of *Phoenix dactylifera* L. (Date Palm) for Combating Desertification and Enhanced Livelihood: Nacgrab R and D Focus

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Keywords: *Phoenix dactylifera* L, germplasm, in vitro, TIBs

Abstract

Nigeria is a country with diverse landscapes and climatic conditions that result in a corresponding high diversity of biological niches harbouring many plant species. The country is equally endowed with several ecological zones, having on its far south mangrove/swamp while the far north is defined by its almost desert-like climate. Most of the states in this axis are Jigawa, Bornu, Kebbi, Yobe, Sokoto, Katsina and Zamfara. The vegetation cover of these areas is mostly Sudan savannah and Sahel savannah and the desert encroachment in these front line states is so fast and growing at an alarming rate. The resultant effect of this has been mass displacement of inhabitants, farms and their animals thus inflicting hardship and poverty. Meanwhile, studies have shown that few tree crops do relatively well in these areas and one of them identified is *Phoenix dactylifera* (date palm). Date palm has high nutritive and commercial value and plays an important role in the ecology of various desert and semi-desert environments as well. Date palm, which is an irreplaceable tree in irrigable desert lands, provides protection to under-crops from the harshness of the climate (heat, wind and even cold weather), reduces damage caused by sand storms and wind erosion. It is therefore noted with keen research interest that despite the huge potentials of the date fruit the availability of planting materials has been the major challenge of the cultivation and production of this very important desert crop due to the heterozygous and dioecious nature of the plant. The National Centre for Genetic Resources and Biotechnology (NACGRAB) - the national focal point on genetic resources conservation and utilization - in one of her recent germplasm exploration and collection exercises in the affected front line states, is collecting several accessions of dates which could be subjected to in vitro propagation techniques using shoot tips and embryos in a modified Murashige and Skoog medium containing adenine, naphthalene acetic acid (NAA) and activated charcoal. The generated plantlets could be subcultured into a liquid multiplication media using the Temporary Immersion Bioreactor systems (TIBs). The resultant products are expected to have a higher multiplication quotient than when the conventional solid multiplication media are used, thereby increasing the availability of planting materials for date palm estate establishment in Northern Nigeria.

INTRODUCTION

Nigeria is situated on the West African coast with a land mass of 923, 768 km^2 which is characterised with diverse landscapes and climatic conditions that result in a corresponding high diversity of biological niches harbouring many plant species. Out of this, 600 meters of the arable landmass is lost yearly to fast desert encroachment (www.thisdayonline.com, 26 January 2009), especially, in the northern region which is characterised by the Sudan and Sahel savannah vegetation types, thus displacing the inhabitants, farms and their animals and thereby inflicting hardship and poverty, and eventual threat to the country's food base. Recent studies have shown that few tree crops do relatively well in these areas and one of them identified is *Phoenix dactylifera* (date

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palm).

Date palm, *Phoenix dactylifera* L. (Family: *Palmaceae*), is a monocotyledonous, dioecious palm, and is considered the most important fruit tree in many Arab countries, such as Saudi Arabia and Iraq (Badawy et al., 2005). The Date palm is a very beautiful, elegant and tall palm. It grows slowly, about 1 foot a year to a height of 80-100 feet and can live for more than 200 years. Only a female tree can produce dates. Usually it starts producing fruits after 5-8 years. The date fruit consists of 70% carbohydrates (mostly sugars), making it one of the most nourishing natural foods available to man (FAO, 2002). The flesh of dates contains 60 to 65% sugar, about 2.5% fibre, 2% protein and less than 2% each of fat, minerals, and pectin substances. Date fruits are also a good source of iron, potassium and calcium, with a very low sodium and fat content thus making it good for anaemia treatment. In addition, moderate quantities of chlorine, phosphorous, copper, magnesium, silicon and sulphur are also found in the date fruit (FAO, 2002). Seeds may be ground for animal feed and the oil is used in soap and cosmetics. The date palm tree leaves are used for basketry and wickerwork. The leaves may be used for making huts while the leaves fibres may be made into nets. The trunk may be used for timber works as well as fuel. The trimmed fruit stalks are used as brooms. They are also used for making ropes and belts. The high tannin content of the fruit can also provide medicinal benefits to man, like laxative food and treatment of constipation.

The selenium in date fruit helps enhance the immune system and also lowers the risk of cancer and heart diseases. The date palm syrup and infusion is a good remedy for cough, fever, flu and bronchial catarrh. The roots of the date palm are used to fight toothaches. Dates are a very good food source for babies. It is an effective medicine for diarrhoea and dysentery during teething time.

All the above enumerated minerals and benefits are the basic ingredients needed for the physical, mental and social development of man. In terms of commerce both national and international trade in dates are very impressive, FAOSTAT 2002, reported that the world average export trade stands at US$ 258 million as at year 2000 with countries in the middle east dominating the world market. In addition to the dates' high nutritive and commercial value it is also one of the main trees used as ornamental and in landscaping (Badawy et al., 2005). The date palm could play an important role in the ecology of various desert and semi-desert environments. Date palm, which is an irreplaceable tree in irrigable desert lands, provides protection to under-crops from the harshness of the climate (heat, wind and even cold weather), reduces damage caused by sand storms and wind erosion.

Tissue culture is a technique mainly used for rapid propagation of several perennial fruit trees, including date palm (Dass et al., 1989). However, adequate availability of planting materials has been the major challenge of the cultivation and production of this very important desert crop in Nigeria. Plant in vitro regeneration is a biotechnological tool that offers a potential solution to this problem as it provides a means of putting the plants on the market at lower prices.(Afolayan and Adebola, 2004). Problems of planting materials and propagation of date palm arise from the fact that the tree has a long life cycle (Ammar and Badeis, 1983), and that the number of offshoots produced by them is limited to a certain period in the life of the tree (Barret, 1973). Also, the tree is dioecious and heterozygous. Abo El-Nil (1986) and Al-Ghamdi (1993). The success of date palm cloning by tissue culture methods, based on organogenesis and somatic embryogenesis, has been investigated by many workers. Organogenesis in date palms has a low efficiency due to the low number of explants that form plantlets in vitro, the long time required for the initiation phase, the low multiplication rate and the strong influence of the variety (Poulain et al., 1979; Beauchesne, 1982). Somatic embryogenesis has been obtained from shoot tips which were excised from offshoots (Tisserat, 1979, 1982; Sharma et al., 1984, 1986, 1991; Zaid and Tisserat, 1983; Mattar, 1986a; Daguin and Letouze, 1988; Diwaker et al., 1998; Djamila and Bougedoura, 1988), with the resulting embryo regenerating into plantlets (Bekheet et al., 2000; Badawy et al., 2005).

To meet the increasing demand for date palms, it is necessary to complement the

tissue culture techniques with Temporary Immersion Bioreactor system (TIBs) to enhance the commercial production of date palm seedlings.

Mass propagation of plants by tissue culture is labour intensive and costly; this therefore calls for a need to advance on the technique. TIBs is the use of liquid medium for in vitro culture and it is a relatively recent micro-propagation procedure that allows connotation increase of multiplication and automation quotient. It is an automated micro-propagation process through the use of bioreactors which have been designed to provide maximum opportunities for monitoring and controlling environmental condition, immersion time, i.e., duration or frequency, which is the most critical parameter for system efficiency.

TIBs is comprised of two glass flasks of variable capacity, one for the growth of the explants and another as deposit of the culture media. These flasks are connected by silicone tubes by means of the connectors (either 'T', 'L' or straight/parallel). In the second connector of each flask a similar tube is placed with a hydrophobic filter of 0.22 micron in the other end of these tubes, to guarantee the sterility of the air. In the internal part of each cover two tubes are also placed to one of the connectors, one that has as function to extract the culture media in both recipients. The means of circulation from a flask to another dependence on the opening or closing of the electro-valves (solenoids), which are connected to a programmable timer to regulate the frequency and the duration of the immersion. The pressure of air is regulated by a gauge coupled to the air compressor, and also controlling its automatic ignition.

The plants are exposed to the liquid medium intermittently rather than continuously and as the plants are not always in contact with the medium, nutrients absorption and growth rate are thus stimulated, therefore it has greater feasibility of producing higher plantlets volume.

Temporary immersion systems for plant micro-propagation can be described and grouped into 4 categories according to operation: i) establishment of the plant on a solid medium, machines, ii) partial immersion of plant material and renewal of nutrient medium, by gradual reduction of the agar concentration, iii) partial immersion on a liquid nutrient renewal mechanism, iv) complete immersion by pneumatic driven transfer of liquid medium and without nutrient medium renewal. It has also frequently been considered an ideal technique for mass production as it reduces production costs, followed by a reduction in shelving area requirement and the number of containers used; manual labour and facilitates changing the medium composition. Complete atmosphere renovation inside the recipient at regular intervals, which means there is no large accumulation of gases like ethylene. Agitation due to air flow during the immersion phase causes scattering of vegetative tissues.

Plant material propagated by temporary immersion performs better during the acclimatization phase than material obtained on semi-solid or liquid media. Hyperhydricity, which seriously affects cultures in liquid medium, is eliminated with these culture systems or controlled by adjusting the immersion times, quite little immersion time, in which most of the tissues are covered with a film of media.

Figures 1-5: TIBs shelve, showing the principal components, timers, bottles, and solenoids valves.

Thus, the aim of this study is to investigate how to complement the tissue culture techniques with the TIBs for commercial production of date palms seedlings for possible establishment of its estates in the front line states through participatory approach with the farmers and the communities.

MATERIALS AND METHODS

This study will be conducted at the Tissue Culture Lab of the National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Nigeria. It will involve the collection of offshoots of date palm from across its geographic range.

Plant Material

Two-year-old offshoots of *Phoenix dactylifera* will be carefully separated from their mother plants (female date palm) when they will be 60-80 cm tall, with 8-12 leaves and used as a source of explants. The shoot tip and axillary bud will be the explants to be used.

Preparation of Explants

1. Sterilization and Culturing of Explants. In the laboratory, offshoot leaves will be removed acropetally till the tender portion will be reached. It will be further trimmed to completely remove the woody tissues and keep the succulent shoot-tip intact. The tips obtained will be kept in an anti-oxidant solution .The date palm shoot tips (1-2 cm long, with a diameter of approximately 0.5-1.0 cm), including the apical meristem will be excised and cut into 4. The explants (the tips and axillary buds) will be washed with liquid detergent under running tap water, and then it will be surface sterilized by immersing in 70% ethanol for 5 min and in a solution of sodium hypochlorite (20% V/V) containing few drops of Tween 20 for 20 min. This will be followed with rinsing thrice in sterile distilled water. Then, using a simple microscope under a lamina flow hood, the young leaflets and part of the core's tissue will be removed gradually till reaching the top bud and will be inoculated on a Murashige and Skoog (1962) medium (MS).

Culture Media

The initiation media consist of MS medium containing 100 mg/L 2, 4-D + 3 mg/L 2ip. The pH of the medium will be adjusted to 5.7 before the addition of agar. The culture medium will be distributed into culture jars (150 ml), each containing 25 ml of the prepared medium. The jars will be covered and autoclaved at121°C under a pressure of 0.15 psi for 20 min. The shoot tips and axillary buds (explants) will be inoculated for a period of 18 weeks to obtain callus formation, as reported by (Badawy et al., 2005). The callus obtained will be transferred into a medium without hormones and activated charcoal to enhance embryo production and development. Tisserat (1979), using sucrose concentration of 30 g/L, and incubating the explants on the culture medium for 24 weeks for the formation of somatic embryo and subsequently plantlets regeneration from the embryogenic callus (Bekheet et al., 2000). These developed shoot and buds will then be transferred into TIBs in a liquid medium under a control condition for further multiplication.

The shoots will be regenerated and rooted on a cultured medium containing 40 g sucrose/L, 9 g/L charcoal (AC) and 1.5 mg/L of NAA for a period of 18 weeks. The rooting of date palm was favourably influenced by the presence of activated charcoal with an increase in the number of roots and root length.

Acclimatization and Establishment.

The rooted plantlets will be acclimatized under greenhouse conditions. The plantlets will be rinsed in water to remove the adhering agar. These will be planted in prepared moist soil medium consisting of sterile peat and vermiculite mixture in small perforated polythene bags. These will be transferred to the humidity chamber for hardening. The hardened plants will be transferred to the nursery for some weeks before establishment on the field.

Expected Results and Conclusion

Availability of planting materials is usually faced by the farmers in the adjoining communities in the front line states.

The farmers will also be trained in the seedlings handling, plantation establishment, sustainable fruits harvesting and marketing thereby addressing both the desertification menace and poverty alleviation

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Figures

Fig. 1.

Fig. 2.

Fig. 3

Figs. 1-3. Growing plantlets submerged in liquid medium.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Figs. 4-7. Growing plantlets submerged in liquid medium.

Effects of Time of Pollination and of Pollen Source on Yield and Fruit Quality of 'Najda' Date Palm Cultivar (*Phoenix dactylifera* L.) under Drâa Valley Conditions in Morocco

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Keywords: date palm, pollination time, metaxenia, fruit set, fruit quality, yield

Abstract

In its strategy of intensifying and extending date palm cultivation, Morocco has succeeded to select and micro-propagate new date palm genotypes known by their high fruit quality and Bayoud disease resistance. 'Najda' (INRA-3014) is one of these highly interesting selected genotypes that were distributed as vitroplants to farmers. However, as a newly introduced cultivar, most growers still lack suitable agricultural techniques for its cultivation. The determination of 1) the period at which the female flowers of 'Najda' date palm remain receptive for pollination and 2) the most suitable pollinator to induce a metaxenia effect is very important to date palm growers. For this objective, six 'Najda' palm trees, 15 years old and grown in the same environment of the Zagora Date Palm Experimental Station, were carefully chosen and used to study the effects of 3 polliniser cultivars including: the Moroccan selected males 'NP3' and 'NP4', and a normal male usually used by local growers. Another six palm trees were used for the study of the effect of pollination time and were therefore pollinated at 1, 7, 10 and 15 days from female spathe opening. Both experiments were established in a completely randomized block design. Obtained results showed that pollination by 'NP3' or 'NP4' pollen enhanced fruit maturity by 10 days, and significantly improved fruit length by 1 cm, fruit width by 3 mm and fruit weight by 35% compared to the control. Furthermore, pollinating 'Najda' flowers between the 7th and the 10th day after spathe opening induced significant increase in the mean fruit set by 70% while the mean fruit weight remained acceptable (13,14 g). Pollinating earlier or after this period, significantly reduced fruit set which in turn resulted in somewhat higher fruit characteristics. Based on these results, it seems reasonable to conclude that the 'Najda' cultivar should be pollinated using 'NP3' or 'NP4' pollen, particularly between the 7th and the 10th day after spathe opening.

INTRODUCTION

Date palm pollination plays a key role on the quantity and quality of the produced dates, and therefore its control is of paramount importance. To achieve adequate pollination, some main conditions must be considered, such as:

a) The knowledge of the period of female flowers receptivity to pollen grains. The duration of this period varies with cultivars, so that pollination outside this period will not lead to fertilization and consequently results in heavy drop of unfertilized fruits. The period of receptivity generally reaches its optimum at 3 to 4 days from female spathe opening, and may extend up to 13 days depending on the varieties and the climatic conditions (Zaid and Arias, 2002; Sedra, 2003). After the spathe opening, inflorescences are first white-cream (stage 1), and due to their gradual exposure to sunlight, they turn to a yellow-cream color (stage 2), and finally to a green color (stage 3). In Morocco, growers usually pollinate the majority of their varieties in stage 2 that is during the week following the opening of the spathe. Some varieties such as

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'Bousekri' and 'Aguellid' do not pollinate until the 3rd stage, which usually begins 10 to 12 days after the opening of the spathes. Varieties pollinated at the 1st stage (during the first day after the opening of the spathe), are rare.

b) The use of pollen from a male palm with important characteristics such as the production of the necessary quantity of pollen grains with high fertilization ability and having metaxenic/xenic effects on fruit quality and maturity. This phenomenon of metaxenia has been defined by Swingle (1928), as the effect of pollen on the morphological and physiological characteristics of fruit tissues and seed. Studying these effects in the date palm, Nixon (1955), has selected a group of male palms trees, among which two palms showed extreme metaxenic/xenic effects: 'Fard No.4', which induces a remarkable precocity associated with a relatively small fruit size, and 'Mosque' which, on the contrary, produces large fruits but with late maturity.

The time of floral receptivity and the source of the pollen grains are important factors to consider when choosing a combination of varieties for the creation of a new date palm plantation. These factors are also to be considered during any introduction of new cultivars, in order to prevent a future improper pollination technique that will result in excessive unfertilized fruits drop and consequently low yields.

This is the case of Morocco that has succeeded to select and micro-propagate new date palm genotypes known by their high fruit quality and resistance to Bayoud disease (*Fusarium Oxysporum albedinis*). 'Najda' is one of these highly interesting genotypes that was distributed as vitroplants to farmers (Sedra, 1993; Zirari, 1998; Zirari and Outlioua, 2003). As a newly introduced cultivar, most growers have not yet mastered its pollination requirements. The present investigation was carried out to solve this situation and aims therefore to determine:

1) The period at which 'Najda' female flowers remain receptive for pollination.
2) A suitable pollinator to induce a metaxenia/xenia effect on 'Najda' fruits.

MATERIALS AND METHODS

The present investigation was carried out at the INRA Date Palm Experimental Station in Zagora, located in the Drâa Valley in the South East of Morocco. Twelve 'Najda' palm trees, 15 years old, were carefully selected and all their spathes were bagged using kraft paper bags until their use for the experiment. All the selected palm trees undergo the same cultivation practices applied in the Experimental Station. Because of the unavailability of a sufficient number of palm trees with sufficient number of inflorescences, we were obliged to undertake this field experiment in two separate trials.

Trial No. 1: On the Effect of Pollen Source

Six 'Najda' palm trees were used in this trial and their inflorescences were pollinated as follows:

- 2 inflorescences per palm tree were pollinated with pollen from the selected male palm 'NP3'.
- 2 inflorescences per palm tree were pollinated with pollen from the selected male palm 'NP4'.
- 2 inflorescences per palm tree were pollinated with a pollen generally used by farmers of the region (Control).

All inflorescences were pollinated at the intermediate stage 2 that is a week after the opening of spathes. With the 3 above treatments, the trial was established in a Randomized Complete Block Design containing 3 blocks (two palm trees each) and 2 individuals (inflorescences) per experimental unit.

Dates were harvested at 4 successive periods: 1st, 10th, 20th and 30th of September, as well as the 10th of October of the season. Measured observations concerned the percentage of mature dates in each stalk as well as the length, width and the weight of the fruit. Averages of these parameters were calculated from data of random samples of 100 dates.

Trial No. 2: On the Effect of Time of Pollination

Six 'Najda' palm trees were used in this trial and their inflorescences were pollinated as follows:

- 2 inflorescences per palm tree were pollinated at the 1st day after the spathe opening (stage 1).
- 2 inflorescences per palm tree were pollinated at the 7th day after the spathe opening
- (stage 2).
- 2 inflorescences per palm tree were pollinated at the 10th day after the spathe opening (stage 3).
- 2 inflorescences per palm tree were pollinated at the 15th day after the spathe opening (stage 4).

Pollen from 'NP3' selected male palm was used in this trial. With the 4 above treatments, the trial was established in a Randomized Complete Block Design containing 3 blocks (2 palm trees each) and 2 individuals (inflorescences) per experimental unit. Fruit harvesting was performed on September 20th of the season. Collected observations concerned the mean percentage of fruit set and the average fruit weight, calculated from data of random samples of 100 dates.

Statistical data analysis of both trials was performed using the ANOVA test at 5%, while the means comparison was performed using Fisher's least significant difference at 5%.

RESULTS

The present field investigation showed that both pollen source and time of pollination significantly affected the maturity, the dimensions (length and width), and the weight of the produced dates.

Effect of Pollen Source

By using the control pollen, the percentages of mature dates from each of the five harvests times (1st, 10th, 20th, 30th of September and 10th October) were always lower than those obtained with 'NP3' or 'NP4' pollen, and that no significant differences were obtained between these two pollens (Fig. 1). Stalk pollinated with 'NP3' or 'NP4' pollen, reached full maturity (more than 80% of mature dates) by 20th of September, whereas those pollinated by control pollen have reached this stage after 30th September, that is 10 days later.

The differences obtained at the stalk full maturity were followed by significant differences in the mean sizes and weights of the produced dates. Analysis of Table 1 shows that the lowest fruit dimensions were obtained when pollination was performed with the control pollen. With inflorescences pollinated using 'NP3' or 'NP4' pollen, the average length and width of the produced dates were higher by more than 1 cm and 3 mm respectively, than the control. However, no significant differences were observed between these two types of pollen; 'NP3' and 'NP4'.

Parallel to the obtained improvement in fruits dimensions, there was also a marked increase in the average weight and average flesh/seed ratios of the produced dates. Figure 2 shows that by using the control pollen, the average weight and average flesh/seed ratios were always lower than those obtained when pollination was performed with 'NP3' or 'NP4' pollen, and that no significant differences were obtained between these two pollen types. By using 'NP3' or 'NP4' pollen, we could harvest dates over 16 g of average weight, that is an increase in fruit weight of about 35% compared to the control. In addition, dates from pollination with 'NP3' or 'NP4' pollen got flesh/seed ratios between 14 and 16, which is an improvement of about 28% compared to the control.

Effect of Time of Pollination

The analysis of Table 2 shows that the fruit set percentages and the average weights of produced dates vary depending on the time of pollination. The highest fruit set percentages, 70.27 and 69.23%, were obtained when pollination was performed at the 7th

and 10th day after the spathes opening respectively. Concerning the average fruit weight, the greatest values, 14.18 and 14.34 g, were obtained when pollination was performed at the 10th and 15th day after spathes opening respectively.

DISCUSSION

The present investigation clearly demonstrated that a significant improvement in dates maturity and size can be achieved through the control of the pollination technique. Our results showed that the maturity and size of 'Najda' date palm cultivar fruits can be significantly improved through the use, at pollination time, of pollen from 'NP3' or 'NP4' selected male palms. Stalks pollinated using these pollens, reached 100% maturity 10 days earlier than those pollinated by the control pollen. Average length and width of the produced dates increased by more than 1 cm and 3 mm respectively, while their average weight increased by more than 35% and their flesh/seed ratio increased by almost 28%. Dates harvested from stalks pollinated with 'NP3' or 'NP4' pollen were clearly larger and fleshier than those from the control. Such results are comparable to those reported by Nixon (1955), Peraeau-Leroy (1958) and Sedra and Zirari (1998).

The present results also showed that the dates size of 'Najda' date palm can be significantly improved if inflorescences are pollinated at the 7th and the 10th day after spathes opening. Inflorescences pollinated 7 days after the spathe opening resulted in higher rate of fruit set (over 70%) but with relatively lower fruit weights, whereas those pollinated on the 10th day after spathes opening has resulted in greater average fruit weight (over 14 g) however with lower fruit set. These results confirm the fact that good fruit set often results in lower fruit weight, while a low fruit set would generally lead to better fruit weight. Consequently, it became necessary to find a reasonable balance between the fruit set and the average weight of produced dates. To this end, it seems reasonable to conclude that for the Moroccan 'Najda' date palm cultivar, the best results for both fruit set percentage and average weight/size of produced dates can be obtained when pollination is performed between the 7th and the 10th day after spathe opening. Such outcomes on 'Najda' cultivar are similar to those obtained by Chafik (1985) on the 'Mejhoul', 'Tademmamt' and 'Aguellid' varieties.

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Tables

Table 1. Average length and width of produced dates according to the pollen source. Values of the same column followed by different letters are significantly different (LSD at 5%).

Pollen source	Mean fruit length (cm)	Mean fruit width (cm)
Control	3,41 a	2,29 a
NP3	4,32 b	2,55 b
NP4	4,42 b	2,68 b

Table 2. Fruit set and average fruit weight depending on time of pollination. Values of the same column followed by different letters are significantly different (LSD at 5%).

Pollination time (days after spathe opening)	Fruit set (%)	Mean fruit weight (g)
1	49,32 a	12,81 a
7	70,27 b	12,11 b
10	69,23 b	14,18 c
15	39,60 c	14,34 c

Figures

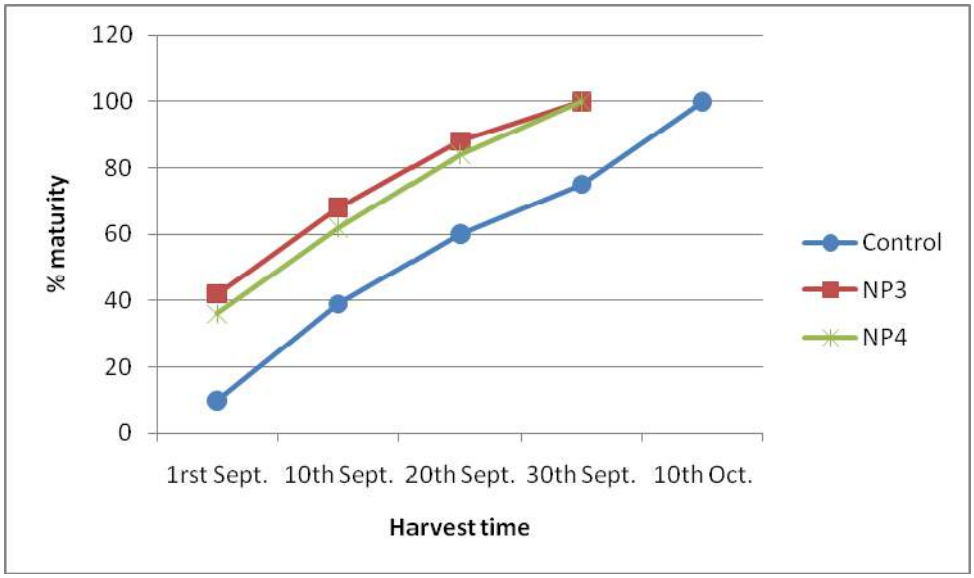

Fig. 1. Percentage of stalk maturity as affected by pollen source and harvest time.

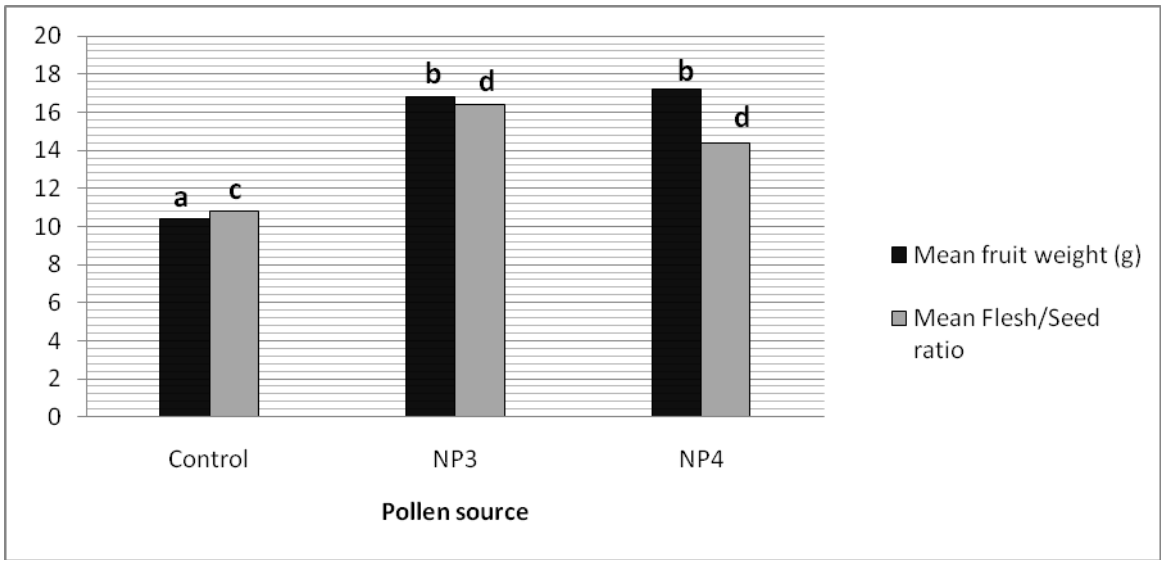

Fig. 2. Averages of fruit weight and flesh/seed ratio as affected by the pollen source. Values of columns of the same color and followed by different letters are significantly different (LSD at 5%).

Use of Bio-Pesticide - New Dimension and Challenges for Sustainable Date Palm Production

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Keywords: Integrated Pest Management, *Phoenix dactylifera*, Cosmopolitan, Agricultural development, Arab world, *Rhyncophorus ferrugineus* Oliver

Abstract

According to the WHO, 1990 report, 25 million cases of acute occupational pesticides poisoning happen in developing countries each year. The environmental hazards resulting from half a century's intensive use of synthetic organic crop protection agents makes it imperative to consider alternative or complementary approaches to sustainable agricultural development and integrated pest management.

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world and is mentioned 20 times in the Quran and several times in the Bible. The number of the date palms is about 100 million worldwide, of which 62 million palms can be found in the Arab world. The aim of this study was to identify and evaluate suitable and new plant extracts with eco-friendly activities against endoparasitic Larval Red Palm Weevil, *Rhynchophorus ferrugineus* Oliver, found in date palm trees (*Phoenix dactylifera* L). These weevils are so effective that even a single pest if present destroys the tissues of the trunk of the tree, resulting therein the falling of the palm tree. Biopesticides of plant origin could be the key to the future. Biopesticides, as aware, are an important group of pesticides that can reduce pesticide risks having a narrow target range and a very specific mode of action it suppresses, rather than eliminates, a pest population.

Biopesticides are inherently less harmful than conventional pesticides. Biopesticides are designed to affect only one specific pest or, in some cases, a few target organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals. Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. Biopesticides play an important role in providing pest management tools in areas where pesticide resistance and environmental concerns limit the use of chemical pesticide products. Keeping in view the above fact ZCHRTM worked on a Biopesticide Management Program.

INTRODUCTION

The date palm is believed to have originated in the lands around the Persian Gulf and in ancient times was especially abundant between the Nile and Euphrates rivers. The date has been traditionally a staple food in Algeria, Morocco, Tunisia, Egypt, the Sudan, Arabia and Iran. The founder of the UAE attached a great importance to agricultural development in general, and to the date palm in particular. This attention is clearly evident in the fast growth in the number of palm trees in the continued increase in the size and variety of date products, in the extensive use of modern technologies, and in the important initiatives undertaken in the areas of manufacturing and marketing of date fruit.

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Over and above it is one of the 20 plants and vegetables discussed in Holy Quran. The plants mentioned in the Holy Quran have special significance not only because of their properties and uses but also on account of their relevance to the events and happenings associated with them.

Quranic Name : Al Nakhl; Al-Nakhil
Botanical Name : *Phoenix dactylifera* Linn. (Family *Arecaceae*)
Common Name : date palm

Quranic References

1. SURA II (Baqara the Heifer). V:266
2. SURA VI (Anam-cattle). V:99
3. SURA VI (Anam-cattle). V:141
4. SURA XIII (Raad-Thunder). V:4
5. SURA XVI (Nahl-The Bee). V:11
6. SURA XVI (Nahl-The Bee). V:67
7. SURA XVII (Bani Israel-The children of Israel) V:91
8. SURA XVIII (Kahf-the cave) V.32
9. SURA XIX (Maryam-Mary). V:23
10. SURA XIX (Maryam-Mary). V:25
11. SURA XX (Tatta-Mystic Letter, T.H) V:71
12. SURA XXIII (Mu-min-The Beleivers). V:19
13. SURA XXVI (Shu araa-The Poets). V:148
14. SURA XXXVI (Yasin-The abbreviated letters). V:10
15. SURA L (Qaf-Abbreviated letters). V:34
16. SURA LIV (Qamar-The Moon). V:20
17. SURA LV (Rahman-God most gracious) V:11
18. SURA LV (Rahman-God most gracious) V:68
19. SURA LXIX (Haqqa-The sure reality). V:7
20. SURA LXXX (Abasa-He frowned). V:29

Medicinal Uses

Besides its food value, the fruit, because of its tannin content, is used medicinally as a detersive and astringent in intestinal troubles. In the form of an infusion, decoction, syrup or paste, is administered as a treatment for sore throat, colds, bronchial catarrh. It is taken to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles. And it is said to counteract alcohol intoxication. The seed powder is an ingredient in a paste given to relieve ague. A gum that exudes from the wounded trunk is employed in India for treating diarrhea and genito-urinary ailments. It is diuretic and demulcent. The roots are used against toothache.

Red Palm Weevil

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Oliv., also called the Indian palm weevil, is well known in the Middle East where it causes severe damage on date palms (Table 1). The RPW was first noted in the Arabian Peninsula in the mid 1980s and in Egypt in 1992. The weevil was first observed in Ras El Khaima, United Arab Emirates in 1985. Approximately, 5 to 6% of palms in the Middle East region are infested with the RPW with an annual rate of infection of about 1.9 (Table 2).

The rate of infestation is about 2.02 ($1300 \times^{5} = 44000$) and about 1.70 ($1000 \times^{9} = 120{,}000$) for the United Arab Emirates (UAE) and the Kingdom of Saudi Arabia (KSA), respectively. The average rate of annual infestations could be 1.9. (Infestation year n=infestation year (n-1) × 1.9). The RPW was wrongly classified as a coconut pest. Indeed, as early as 1970, the RPW was found in India attacking date palms (Khawaja and Akmal, 1971). The first warning came from Dr. Djerbi (1983) who was the first to realize the danger and to invite date growing countries to conduct studies on the biology of this pest, and on appropriate control measures. According to Dr. Oehlschlager (1998), there

are five species of palm weevils in the genus *Rhynchophorus* that are economically damaging to palms (Table 3). Up to December 1998, the following countries are officially declared as having the RPW infestation: Australia, Burma, China, Egypt, India, Indonesia, Iran, Iraq, Malaysia, Pakistan, Papua New Guinea, Philippines, Saudi Arabia, Sri Lanka, Taiwan, Thailand, Tanzania, UAE and Vietnam. According to Prof. Zaid (1999), three more countries are added to the above mentioned list (Jordan, Israel and Palestine): On April 21,1999, Prof. Zaid identified by e-mail scanning, the photo of the first red palm weevil found in Jericho (Palestine). On May 6, the weevil was found in Jordan (in Shunae), a few kilometres north-east of Jericho. On May 14, another weevil was found in Israel, along the Jordanian border at Moshav Yafit (15 km north of Jericho).

The red palm weevil is one of the most destructive and dangerous pests for date palm in Asia and the Middle East. It has caused mass destruction in palm-tree plantations in Egypt and the Gulf countries. It also attacks coconut trees, sugar cane, and various ornamental trees. The red palm weevils are large insects (greater than 25 mm long). They are found over a very wide geographical area involving different climates. This pest lays its eggs on the bark of the palm tree; the grubs eventually hatch, drill into the trunk and eat the entire inside of the tree. Under heavy attack, the tree weakens and inevitably dies. The weevils are attracted to dying or damaged parts of palms, but also attack undamaged palms. The damage is caused by the larvae that bore through the soft tissue such as the tree crown, the upper part of the trunk and at the base of the petioles. On average, females lay 260 eggs which take 3 days to hatch. The larval period takes two months and the pupal period, three weeks. These figures vary across different regions. The females use the rostrum to bore into the plant tissue to form a whole in which they lay their eggs. This occurs most frequently in crowns which have been damaged.

Normally, the Red Palm Weevil prefers to infest palms below the age of 20 years, where the stem of the young palm is soft, juicy and easily penetrated. The weevils are destructive pests to palms. The larvae are responsible for damaging the palm, and once they have gained access, the death of the palm generally ensues. The larva normally never comes to the surface, since it begins its life inside the palm. Therefore, neither the damage nor the larva can be seen. However, the trunk of the palm can be infested in many parts, including the crown. The damage caused by a few larvae of the weevil is astonishing. Even one larva may cause considerable damage, and, sometimes the death of the palm. It is difficult to assess the actual loss caused by this pest, but undoubtedly it affects the production of date palms.

Control

The challenge is to detect the presence of the weevil early enough so the tree can be saved. There are no external signs that would indicate that a tree has been infested; the leaves remain green up to the moment the tree falls over, its insides devoured. The thumb-sized grubs make so much noise eating that if you stand next to an infested tree you can actually hear them munching their way through the trunk. Another sign of infestation is a red gelatinous substance which has a putrid odor and which leaks out as the grubs feed. But by the time the smell can be detected by humans, it is too late for the tree: it must be cut down and burnt, otherwise the larvae will begin attacking nearby trees. In the Gulf region agriculture experts are testing a new method of fighting the red palm weevil using a harmless fungus: the Brazilian bioactive fungus which may be an effective deterrent because it has the ability to destroy the pest in the larval stage.

Another method involves pheromones which attract the red weevil, then an insecticide in a bucket attached to the trunk of the tree would kill it. But the problem is only partially solved because the larvae continue to thrive in the trunks of the palm trees and emerge as fully-grown insects. Another strategy involves collecting mature weevils and injecting them with a poison which prevents them from reproducing and results in the death of any female they mate with. They are being implemented in UAE and Saudi Arabia. The Ministry of Agriculture and Fisheries of UAE has introduced Acecap capsules in its Integrated Pest Management (IPM) to fight the red palm weevil. The

Acecap capsules are introduced in small holes in the trunk. The larvae are killed by the active components of the capsule that are absorbed through the trunk (each Acecap capsule contains 875 milligram of active Acephate). Each tree uses between 7 to 10 capsules annually depending on the degree of infestation. A tree can be cured in two months and will stay healthy for a year. Acecap capsules are used in Saudi Arabia, Kuwait and Oman.

The Department of Agriculture and Fisheries of UAE set up a technical committee to help fight the red palm weevil. Its directives include using nematodes to fight the larvae. The nematode is injected into the trunks of infected palm trees. Once inside, they remote-sense the weevils, penetrate into them and then release a deadly bacterium which kills them through blood poisoning within 72 hours. To concentrate the destructive weevils in one place and kill them, the project is using two naturally occurring substances known as aggregation pheromones and kairomones. Pheromones are chemical compounds produced in minute quantities by male weevils which cause them to gather in a hotspot. Kairomones are compounds emanating in tiny quantities from the fresh scars of trees that have been recently pruned. These two compounds are used to lure, trap and kill the weevils in mass quantities. But first the team has to locate the infected palm groves. By using satellite remote sensing and imaging technology they aim to build up a comprehensive picture of the weevils' geographical distribution across Saudi Arabia. Once a weevil infestation is located from the air through color changes, the priority is to eradicate the focus and prevent the pest from spreading to healthy plantations.

The environmental hazards resulting from half a century's intensive use of synthetic organic crop protection agents makes it imperative to consider alternative or complementary approaches to sustainable agricultural development and integrated pest management. Biopesticides of plant origin could be the key to the future. Indeed, in the past thirty years, advances in analytical chemistry and molecular biology have led to a better understanding of the interactions between plants and pests, and the communication mechanisms between organisms.

Biopesticides

Biopesticides are inherently less harmful than conventional pesticides. Biopesticides are designed to affect only one specific pest or, in some cases, a few target organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects, and mammals. Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.

When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. To use biopesticides effectively, however, users need to know a great deal about managing pests.

Generally, all biopesticides exhibit the following characteristics: narrow target range, highly specific mode of action, suppress pests, do not eliminate, critical timing of application, limited field persistence, short residual effect, safer to environment and safer to people. Their use is best as part of an Integrated Management Program (IPM).

MATERIALS AND METHODS

Azadiracta indica; leaf extract, seed kernel extract.

Annona squamosa; seed extract (Indian), seed extract (Australian).

Capsicum frutenscens; pod extract (pericarp and seeds).

The above parts of all the plants in their vegetative stage were collected from Abu Dhabi, UAE. The specimen of the materials, as specimen Nos.435-440/ZCHRTM, are preserved in the herbarium of the Zayed Complex for Herbal Research Centre, UAE for record. These plant materials were dried under shade and processed using standard methods. The water and 70% alcoholic extracts were prepared using Rotaroy Evaporator-Buchi Rotavapor (R-153) fitted with a chiller. The powdered materials were used for

fluorescence analysis and successive extraction using a soxlet extractor. The quality control studies of the powdered aerial parts of all plants and their water and 70% alcoholic extract were performed using standard procedures from different Pharmacopoeias. To develop the thin layer chromatograms, silica gel $60F_{254}$ precoated glass plates (Merck, Darmastadt) were used. The TLC plates were developed in solvent systems Benzene:Pyridine:Formic acid (36:9:5) and Toluene:Ethyl acetate (93:7), comparative studies were made using authentic samples. The TLC plate image was captured with the CAMAG Videostore 2 (Switzerland) under visible light. For IR fingerprints pellets, prepared by mixing the dried raw materials/extracts with KBr in 1:100 ratio, were scanned in Perkin Elmer Paragon 1000 FT-IR spectrometer in the scan range of 4000-400 cm^{-1}. The UV spectra were recorded on Spectronic Genesis 2 Spectrometer (Milton Roy, USA) fitted with a 8-position multi cell holder. Extraction was performed using Dionex ASE-200 Accelerated Solvent Extractor (USA). For HPLC studies a Waters 600E analytical HPLC attached with UV/VIS detector and controlled by Millenium (ver. 2.15) software was used. 100 mg of 10% alcoholic extract was dissolved in 10 ml of distilled water and centrifuged for 10 mins. The supernatant liquid was passed through a Sepak C_{18} cartridge equilibrated with 5 ml of methanol and 10 ml of water. The cartridge was eluted with two 15 ml portions of methanol. All the eluents were pooled together, dried and re-dissolved in 10 µl of methanol. 25 µl of this solution was injected in to the HPLC µBondapak C18 (3.9×300 mm 10 µm 125°A).

Quantitative analyses of the 70% alcoholic extract for different organic constituents were performed using modern methods. The ash of the powdered material was quantitatively analyzed for inorganic chemical constituents using atomic absorption spectrophotometer. For the centrifugal partition chromatography a Pharma-Tech CCC-1000 Instrument was used. Representative morphoanatomic characteristics were assigned by the help of a Leica Microscope equipped with a JVC color video camera connected to a computer and printer.

Trials were conducted in vitro in the lab. Nine petridishes were taken and in each of the petridishes 4 large uniformly sized larval Red Palm Weevils were taken along with some plant fibers. Each petridish was numbered starting from 1 to 9. Each petridish was poured with mixture of plant extract and water in ratio of 3 g of the plant extract to 50 ml water. The larvae were allowed to be in the petridishes filled with trial plant extract water for about 10 min.

Sample contents: Sampling in petridish
Azadiracta kernel + water
Annona squamosa kernel + water
Azadiracta leaf + water
Annona kernel + *Capsicum* + water
Azadiracta kernel + water
Azadiracta + *Annona* + *Capsicum* + water
Dichlorves 50% E.C + water (insecticide)
Water only (control)

Each of the above mentioned plant extract and insecticide is mixed with water at the concentration of 6% (that is 8 g in 50 ml water). If two extracts are mixed then they are mixed 4 g each in 50 ml of water. In the last petridish water alone was kept as a control.

RESULTS AND DISCUSSION

Observation Larvae were allowed to be in the mixture in the petridishes for about 10 min. Then the larvae were removed and were spread on bloating papers separately to wipe out excess plant extract/insect water from their bodies. Then they were observed for the effect of the test so on the larval mortality and the following results were observed.

No	Plant extract	Conc.	Total no. of Larvae	Live
1	*Azadiracta* kernel + water	6%	4	2
2	*Annona* kernel + water	6%	4	2
3	*Annona* + Azadiracta + water	6%	4	0
4	*Azadiracta* leaf + water	6%	4	2
5	*Annona* + *Capsicum* + water	6%	4	3
6	*Azadiracta* kernel + *Capsicum* + water	6%	2	2
7	*Azadiracta* kernel + *Annona* + *Capsicum* + water	6%	4	0
8	Dichlervos + water	6%	4	0
9	Water only		4	4

Inference The mixture, viz. 3-, *Annona* + Azadiracta + water; 7-Azadiracta + *Annona* + *Capsicum* + water and 8-Dichlorvos + water gave 10 larval mortality while others gave mortality in varying degree.

Result *Annona squamosa* and *Azadiracta indica* extracts with or without *Capsicum* extract gave 100% mortality percentage which is also the case of the insecticide Dichlorvos which is a organophosphoric insecticide that can be replaced and substituted with the botanical extracts.

Identified and evaluated suitable and new plant extracts with eco-friendly activities against endoparasitic Larval Red Palm Weevil, *Rhynchophorus Ferrugineus* Oliver were found in date Palm tree (*Phoenix dactylifera* L). Biopesticides of plant origin could be the key to the future sustainable date palm production. The above two plant extracts have been found very effective to kill the aforesaid pest insects: *Azadiracta indica* (Neem) seed and seed kernel and *Annona sqamosa*.

The botanical extracts of *Annona* and *Azadiracta* have promising results in the larval Red Palm Weevil i.e., the in vitro studies in the laboratory showed 100% mortality of these larvae. In vivo studies on infected live trees are under progress to reconfirm this and they are expected to give very encouraging results. Application of these biopesticides will not affect the taste and behavior of the date palm fruits as it is affected by the application of organophosphorous insecticide/pesticides earlier. Further experiments are under progress. This programme has not only national significance but if proved successful, would be applicable worldwide for the sustainable growth of date palm production.

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Zaid, A., De Wet, P.F., Djerbi, M. and Oithabi, A.2002. Date Palm Cultivation, Chapter XII. Diseases ad Pests of Date Palm. FAO, Rome (and the references therein).

Tables

Table 1. Distribution of red palm weevil in the Near East.

Country	First recorded	Area/location infested
Qatar	1985	Doha
UAE	1985	Ras El Khaima
Saudi Arabia	1987	Katiff
Egypt	1992	Salheya, El-Tal El Keber and El-Kassasin
Kuwait	1993	Throughout
Oman	1993	Buraimi, Mahadha, Masandam Governorate

(Source FAO, 1995)

Table 2. Evolution of affected date palms.

	Year		Year	
UAE	1990	1,300	1995	44,000
KSA	1987	Less than 1,000	1996	120,000

Table 3. Rhynchophorus species damaging palms.

Species	Palm hosts	Region
Rhynchophorus ferrugineus	Date	Middle East
Rhynchophorus vulneratus	Coconut	South East Asia
(Same species)	Oil	South East Asia
Rhynchophorus bilineatus	Coconut	Papua New Guinea
Rhynchophorus cruentatus	Sabal	Florida
Rhynchophorus phoenicis	Coconut Oil, Date	Tropical Africa
Rhynchophorus palmarum	Coconut Oil	Central and South America

Source: Oehlschlager, 1998

Figures

Fig. 1. Larva in a date palm trunk

Fig. 2. Adult Red Palm Weevil emerging from its cocoon.

Subsurface Drip Irrigation for Date Palm Trees to Conserve Water

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Keywords: drip irrigation, subsurface drip irrigation, date palm trees, date palm water use, date palm yield, water use efficiency

Abstract

A laboratory and field study was conducted on subsurface drip irrigation systems. In the first laboratory study, eight subsurface drip irrigation lines, available locally, were selected and a number of experiments were made to evaluate lines' hydraulic characteristics to insure their suitability for drip irrigation design requirements with high uniformity and optimum performance, for the purpose to select the best for field experiments. The second study involved field trials on mature date palm trees to study the effect of a subsurface drip irrigation system on the yield and water consumption of date palms, and to compare that with the traditional surface drip irrigation system. Experiments were conducted in the Alwatania Agricultural Project on 50 mature palm trees (17 years old) of the 'Helwa' type with 10 meters spacing between rows and between trees. A high efficiency subsurface line (Tec line) was used based on the results of the first study. Irrigation scheduling was made through a soil moisture sensing device to ensure enough soil water level in the soil. The experimental layout was installed during the 2001 season and measurements continued till the end of the 2008 season.

Results have indicated that there is an increase in the yield and a considerable saving in water compared to the conventional drip irrigation method. In addition there was a high increase in water use efficiency using the subsurface system. The subsurface systems prove to be durable and highly efficient for irrigating date palm trees.

INTRODUCTION

Agriculture is considered the biggest consumer of water resources in the world. The consumption of water by agriculture could account for up to 90% of total annual water consumption in some countries of the arid region like Saudi Arabia. In addition, agriculture could be the reason for water degradation because of the absence of proper water management, therefore, there is an urgent need for optimum use of water for agriculture and an extra emphasis should be directed towards water management to prevent water pollution or deterioration of water quality. In that respect, drip irrigation is considered as one of the most important practical and effective means for irrigation water application compared to other irrigation methods.

The drip irrigation system has proven its superiority over other irrigation systems in increasing yield and reducing the cost of labor and energy, in addition, it improves efficiency and reduces water losses due to evaporation and deep percolation. However, the traditional surface drip irrigation system has some disadvantages including; possibility of damage, exposure of pipe network to sun and the accumulation of salts. A recent better alternative is the subsurface drip irrigation system. It represents the recent improvement in irrigation application, as it prevents or in most cases reduces considerably the evaporation from soil surface and the evapotranspiration is satisfied in a better way due to upward movement of water in the root zone, also the water use efficiency becomes higher as the water is actually added to the active roots, in addition, it prevents the growth of weeds around the crop (Ayers et al., 1995).

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After three decades of research and development, the subsurface drip irrigation system becomes one of the systems that is characterized by high efficiency and productivity. Through extensive research, most of the subsurface drip irrigation system problems were solved including; clogging of emitters by small roots, lateral installation and fertigation. The results of many experiments have indicated a significant increase in efficiency of water and nitrogen use that lead to a high increase in production and an improvement in quality. The system has also contributed in limiting ground water pollution with nitrate and salts in the long run. As the system works under the soil surface, it was noticed that it has an advantage over the traditional surface drip system in saving water and nutrients in addition to the control of salinity, deep percolation and durability of the system, this may be due to the spherical soil water wetting as compared to the half spherical in the case of the surface drip system (Phene, 1995).

Comparing the subsurface drip irrigation system with the traditional surface drip system and the sprinkler irrigation system, results have shown that the overall water use is reduced by 50% compared to the sprinkler system and 30% compared to the traditional surface drip irrigation system. It was also noted that production was increased by a percentage ranging between 30 to 70% compared to the surface irrigation system (Barth, 1995). An experiment on tomato using an automatic scheduling system for subsurface drip irrigation has clearly shown a high saving in water and an increase in yield compared to a non automatic system (Mohammad and Al-Amoud, 1994).

Under the arid and semiarid climates where rainfall is minimal and the air temperature is high, this will result in a high increase in the evaporation rate from the soil surface that results in salt accumulation in the top layer of the soil where active roots concentrate which in turn leads to yield reduction. To minimize salt accumulation caused by evaporation it is possible to apply the subsurface drip system to wash away salts beyond the root zone. This method was applied successfully, on mature pear trees, where subsurface laterals were laid at depths of 30 and 60 cm under the soil surface (Oron et al., 1995). Other experiments on porous subsurface systems have shown that operation pressure has a clear influence on the performance of these pipes, where the best performance was noticed at an operation pressure not less than 80 and not more than 150 kPa (Mohammed, 1998).

Date palm trees are considered one of the most important fruit trees in the Kingdom of Saudi Arabia. The number of trees is more than 23 million and it is increasing every year. The production has increased to reach one million ton in the year 2007, they are grown in an area exceeding 156,000 ha (MOA, 2008). The date palm tree is drought resistant and can withstand salinity up to 4 dS/m without any reduction in yield (Ayers and Wescot, 1985). Although the root zone depth ranges between 1.5 to 2.5 m (Doorenbos and Pruitt, 1977), the tree can take up 65 to 80% of water within a root zone depth not exceeding 1.2 m (Yaacob, 1996). Date palm trees are usually irrigated by a basin irrigation system that uses an abundant amount of water; the quantity is usually decided based on the farmer's experience. The crop water requirement for mature date palms ranges between 115 and 306 m^3, that is equal to 1.15 to 3.06 m^3/ha (Albaker, 1972). If the expansion in date palm agriculture continues at the same present rate in the Kingdom it is expected that a huge quantity of water will be required to irrigate the date palms. However, due to the limited water resources in the Kingdom, it is vital to use some water saving methods such as recent irrigation systems (drip irrigation). Therefore, studies aiming to evaluate the date palm water requirement are essential and will lead to the accurate application of irrigation water with no excessive use.

It is possible to estimate the crop water requirement for date palms based on available information of similar areas such as the studies conducted in the Al-Hassa region in Saudi Arabia (Hussain, 1986; Helal et al., 1986), Egypt (Hussain and Hussain, 1982), and Iran (Furr, 1975). Studies have indicated that low frequency irrigation with high quantities for date palms is better compared to high frequency irrigation (Helal et al., 1986). The results of a study on date palm ('Sakoti') in Egypt on irrigation frequency have shown that the best period between irrigations is four weeks with a quantity equal to

71 mm for each irrigation (Hussein and Hussein, 1982). A study was conducted on the effect of drip irrigation on the growth and yield as compared to the sprinkler irrigation (Reuveni, 1971, 1974), it was concluded that drip irrigation is more superior than the sprinkler system, it was due to the smaller volume of wetted soil under drip irrigation. Results have also indicated that date palms irrigated with drip irrigation show a clear increase in leaves, flowers and fruits compared to those irrigated by a sprinkler system. It was also noted that the yield of drip irrigated palms is higher than those irrigated by sprinkler systems. In a comparison study between traditional drip and bubbler irrigation systems on date palms, it was noted that the accumulation of salts on the surface layer was higher for drip compared to bubbler systems (Naimah, 1985).

Results on experiments of date palm water consumption in the Riyadh area have indicated that the average amounts that have been delivered to date palms per year were; 108 m^3/tree (1.08 m/ha), 216 m^3/tree (2.16 m/ha) and 324 m^3/tree (3.24 m/ha) for corresponding water treatments of 50, 100 and 150% of evaporation rate, respectively (Al-Amoud et al., 2000). Economical analysis of yield for the various irrigation systems (drip, basin and bubbler) in the above experiment have shown that the highest yield was for drip irrigated palms then the basin systems. Differences within water treatments were minimal, in other words, the use of an amount of 108 m^3/year/tree is enough to obtain the highest efficiency of water use for date palms. Comparison between water use efficiencies for various irrigation methods (drip, basin and bubbler) on date palms have shown that the drip system has the highest water use efficiency followed by the basin system then the bubbler irrigation system (Al-Amoud et al., 2000). The optimum date palm response to drip irrigation is due to the nature of the system where water is delivered in a slow process for a relatively long period of time through emitters. This process provides better control and distribution for water through the soil profile to an extent that, losses due to evaporation and deep percolation are reduced to the minimum and therefore, date palm tree can make use of almost all the water delivered.

Economical analysis studies have shown the superiority of the subsurface drip irrigation over the center pivot sprinkler irrigation system. It was found that the total cost for the subsurface drip irrigation system per hectare (including; investment, management, operation, etc.) is less by 30% compared to the center pivot system.

The aim of this research work is to investigate the efficiency and practicality of the subsurface drip system use for irrigating date palm trees and to compare it with the traditional surface drip irrigation system and to study the effect of the subsurface irrigation system on yield and water conservation.

MATERIALS AND METHODS

Laboratory experiments were conducted at the department of Agricultural Engineering Laboratories in the College of Food Sciences and Agriculture, King Saud University, Riyadh. Eight random samples of different types of locally used subsurface drip lines were collected (Table 1), with technical details as shown in Table 2. Various hydraulic trials were made on subsurface pipes using the layout shown in Figure 1. Experiments involved testing flow rates along each line under different pressures to evaluate the distribution uniformity.

Field experiments on date palm trees were conducted in the Alwatania Agricultural Project in Gassim, Saudi Arabia (elevation 649 m, 26°18N; 043°46E). Soil texture is characterized as sandy clay loam soil, with physical and chemical characteristics as outlined in Table 3. The experimental site was selected in the middle of the project, the plot consists of 170 trees of mature trees with distances of 10 meters between trees and between lines of trees. 50 of those trees are selected for study, they are of a popular type known as ‘Helwa’. For irrigating the date palm trees, an irrigation pipe network was designed (Keller and Karmeli, 1975; Howel et al., 1992; Nakayama and Bucks, 1986; Al-Amoud, 1999). A high efficiency subsurface line (Techline) was used based on the results of the laboratory study, it is an integral subsurface dripperline, pressure compensating locally available with pre-installed emitters, 16 mm diameter,

1.125 mm and 3.5 L per meter length in one hour, the hydraulic characteristics are shown in Table 2. All subsurface drip pipes were installed in a circular way around the tree trunk with 3 meter diameter at a depth of about 0.45 meter so that they can feed the active root of the tree. The pipe network included all the necessary units and parts such as; valves, filters, water meters and control board as indicated in the network design layout shown in Figure 2. Irrigation scheduling was made by using soil moisture sensing devices that can measure moisture at a depth of 0.8 meter or lower. The experimental plot is supplied with water from a well in the site with suitable water quality as indicated in Table 4. The area is characterized by high temperature in the summer with low rainfall as shown in the climatic data (Table 5). The system was installed and operated in April 2001 and experiments continued till the end of the 2008 season. Harvesting of crop started at the beginning of August and ended by the middle of September.

RESULTS AND DISCUSSION

A preliminary laboratory trial was made on eight subsurface drip irrigation pipes available locally in the Kingdom, to study their hydraulic characteristics. The aim was to select the most proper pipe to be used for the date palm irrigation experiment.

Laboratory experiment results on drip lines hydraulic characteristics have shown a noticeable effect of operation pressure on the performance of drip lines with different degrees. Figure 3 indicates that, generally, the increase in pressure, will increase flow rate, however, it is evident from the same figure that the fourth drip line (SS4), is the superior in performance compared to the others. The superiority is judged by the lower values of slope curve, or the emitter constant (β). Table 6 shows a comparison of performance for all tested drip lines based on the emitter constant of the flow equation of emitters ($Q = bH^{\beta}$). The study of water distribution along the fourth line (Techline) that was chosen for field experiments indicates that there were minimal fluctuations compared to other lines, possibly, due to manufacturing variations. Although pressures more than 0.5 bar could produce uniform flow distribution along the line, increasing the pressure to more than 1 bar showed better uniformity as shown in Figure 4. As a result of the laboratory experiment the forth drip line (Techline) was selected for better performance and highest uniformity of water distribution. Comparing the uniformity coefficient for the chosen drip line at a practical operating pressure of 1 bar, with standard classifications recommended by The American Society of Agricultural Engineers (ASAE, 2000a, ASAE, 2000b), it shows an excellent level.

Results of field experiments have demonstrated, generally, that there is an increase in yield and decrease in water use for all growing seasons (2001-2008), this resulted in a high increase in water use efficiency (Fig. 5). It should be noted that in the 2000 season, date palms were irrigated by conventional surface drip system, with an average yield of 75.5 kg/tree. In the next year (2001) the new subsurface drip system was installed and operated (at the end of April). By the end of the 2001season, it is noted that water use was close to the previous year but the average yield had increased considerably to more than 120 kg/tree with an increase of 60%. In the second year (2002) the field was totally irrigated by the subsurface drip system adding more water (more than 100 m^3/tree/year), to test the impact of an increased amount of water on yield. The results have shown a high increase in yield that reached to more than 162 kg/tree.

As the main aim of the research is to conserve irrigation water through the subsurface drip system, irrigation for the following years (2003-2008) was reduced, with variable results. The average yield of date palms for these years reached 94 kg/tree/year with an average water consumption of 34.7 m^3/tree/year. The water saving reached 60% with an increase of yield reaching an average of 25%. The true water saving could also be measured through water use efficiency, as shown in Figure 6. It is evident from the figure that there is a high increase in water use efficiency, to almost three-folds, compared to the

conventional surface drip irrigation system.

The difference in water consumption of the subsurface drip system is due to different reasons, including; soil type, soil density, soil structure, organic content, possible emitter clogging. Difference in water use could also be due to fluctuation of operating pressure. It is expected, theoretically, that change in water use will have an impact on yield, however, reduced yield could be due to different factors such as; plant disease, insects, salinity and some other reasons.

In conclusion, the result of this work indicates clearly the positive outcomes resulting from the use of a subsurface drip irrigation system compared to a conventional surface drip system.

CONCLUSIONS AND RECOMMENDATIONS

The laboratory experiment has demonstrated clearly the importance of performance tests for drip lines before field use to insure the high performance of emitters and uniformity of distribution along the drip line. Although the actual performance of drip lines may not match the technical data published by manufacturers, it is important to follow the manufacturer's suggestions regarding the operating pressure. It is noted that, an operating pressure of 1 bar is suitable for most drip lines. It is also noted that turbulent flow drip lines have a better performance compared to other products.

Field experiment results have shown that, water saving could reach up to 60% with an increase of yield that reaches an average of 25%. Subsurface drip irrigation systems have demonstrated high water use efficiency of more than three-fold the conventional surface drip systems. Based on the results of this experiment, it is possible to conclude that subsurface drip irrigation for date palms is an effective and practical method for irrigating date palms where a considerable amount of water could be saved due to the elimination of evaporation water compared to other irrigation systems including the traditional surface drip system. The subsurface systems prove to be durable and highly efficient for irrigating date palm trees. It was noted that the subsurface system could eliminate the weed growth around the tree and prevent salt accumulation on the soil surface.

ACKNOWLEDGEMENTS

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Tables

Table 1. Types of subsurface drip lines.

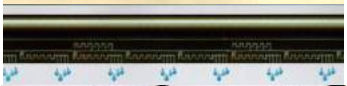
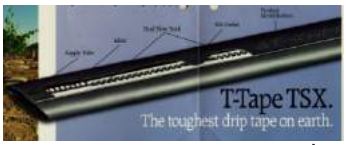
No.	Name	Code	Source	
1	Porous Pipe-1		ECOPORE	
2	In line drippers with filter	GR Dripline System	Eurodrip	
3	In line pressure compensating drip line		Alwassael Co.	
4	In line pressure compensating drip line		Middle East Co.	
5	Tape dripper line	16.5 Drip Size 32	QUEEN GIL	
6	Tape dripper line	T-Tape TSX 508-20-500	T-Tape	
7	Tape dripper line	T-Tape TSX 715-30-340	T-Tape	
8	Porous Pipe-2		ECOPORE	

Table 2. Technical details of drip lines.

No	Code	Drip line	Drip line type	Diameter (mm)	Thickness (mm)	Operating pressure (bar)	Max pressure (bar)	Flow rate (L/hr/m)	No. of drippers/ meter
1	*SS1*	*Ecopore1*	Porous pipe	13	2.2	0.6-1.0	4.0	1.5-2.0	-
2	*SS2*	*Eurodrip*	Inline turbulent flow	17	0.02	0.9	1.4	1.32	3
3	*SS3*	*In-Line*	Inline turbulent flow	13	1				
4	*SS4*	*Techline*	Inline turbulent flow	13	1.1	0.5-2.0	3.9	3.5	3
5	*SS5*	*Queen Gil*	Turbulent tape	16	0.025	1.4-2.0		4.0	3
6	*SS6*	*T-Tape*	Turbulent tape	15.8	0.008	0.4-1.1			
7	*SS7*	*T-Tape*	Turbulent tape	22.2	0.015	0.4-1.1			
8	*SS8*	*Ecopore2*	Porous pipe	16	2.2	0.6-1.0		1.5-2	-

Table 3. Soil analysis results.

Soil Characteristics	Value
Physical characteristics	
Sand (%)	65
Loam (%)	15
Caly	20
Texture	Sandy clay loam
Field capacity	11.2
Wilting point	5.7
Available moisture	5.5
Apparent density	1.62
Chemical characteristics	
Soil pH	7.8
Electrical conductivity (EC) (dS/m)	2.57
Positive ions (Cations) (meq/L)	
Ca	21.3
Mg	9.3
Na	8.4
Negative ions (Anions) (meq/L)	
CO_3	0.22
HCO_3	2.3
Cl	11
Organic matter	0.084
Available elements	
P	6.56
K	152

Table 4. Water analysis.

Water characteristics	Value
Water pH	7.36
Total dissolve salts (TDS)	950
Alkalinity	140
Conductivity	1893
Chlorides	319
Hardness	136
Ca	44
Mg	6.27
Fe	0.026
S	354
NO_3	34

Table 5. Climatic data.

Month	Temperature (°C)	Rel. humidity (%)	Av. Rainfall (mm)	W. speed (m/h)
January	12	41	50	14
February	15	70	80	12
March	19	60	90	12
April	24	55	60	14
May	30	51	20	14
June	32	33	0	14
July	33	20	0	14
August	33	17	0	14
September	31	17	0	9
October	26	19	10	9
November	19	31	50	12
December	15	52	70	12

Table 6. Constants of the relationship ($Q = bH^{\beta}$) which represents the pressure and discharge curves in subsurface irrigation pipes.

No	Code Pipe	b	β	R^2
1	*SS1*	4.170	0.920	0.980
2	*SS2*	7.993	0.543	0.999
3	*SS3*	9.437	0.193	0.966
4	*SS4*	11.441	0.043	0.878
5	*SS5*	4.248	0.610	0.998
6	*SS6*	5.640	0.448	0.897
7	*SS7*	4.665	0.247	0.742
8	*SS8*	44.250	0.950	0.988

Figures

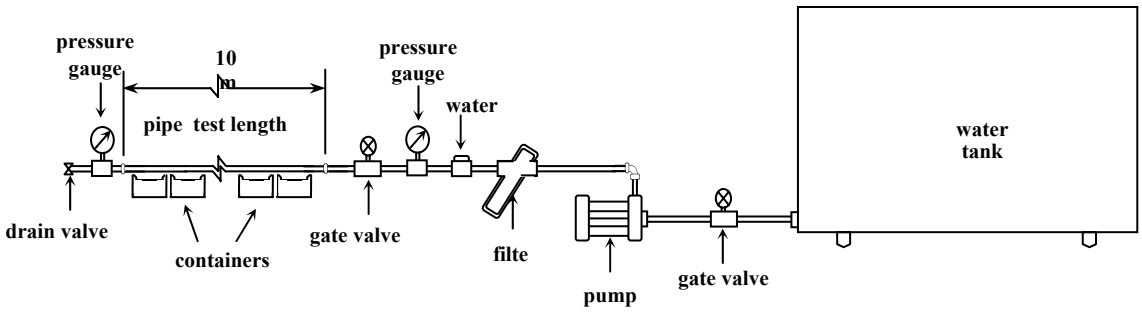


Fig. 1. Laboratory experiment layout.

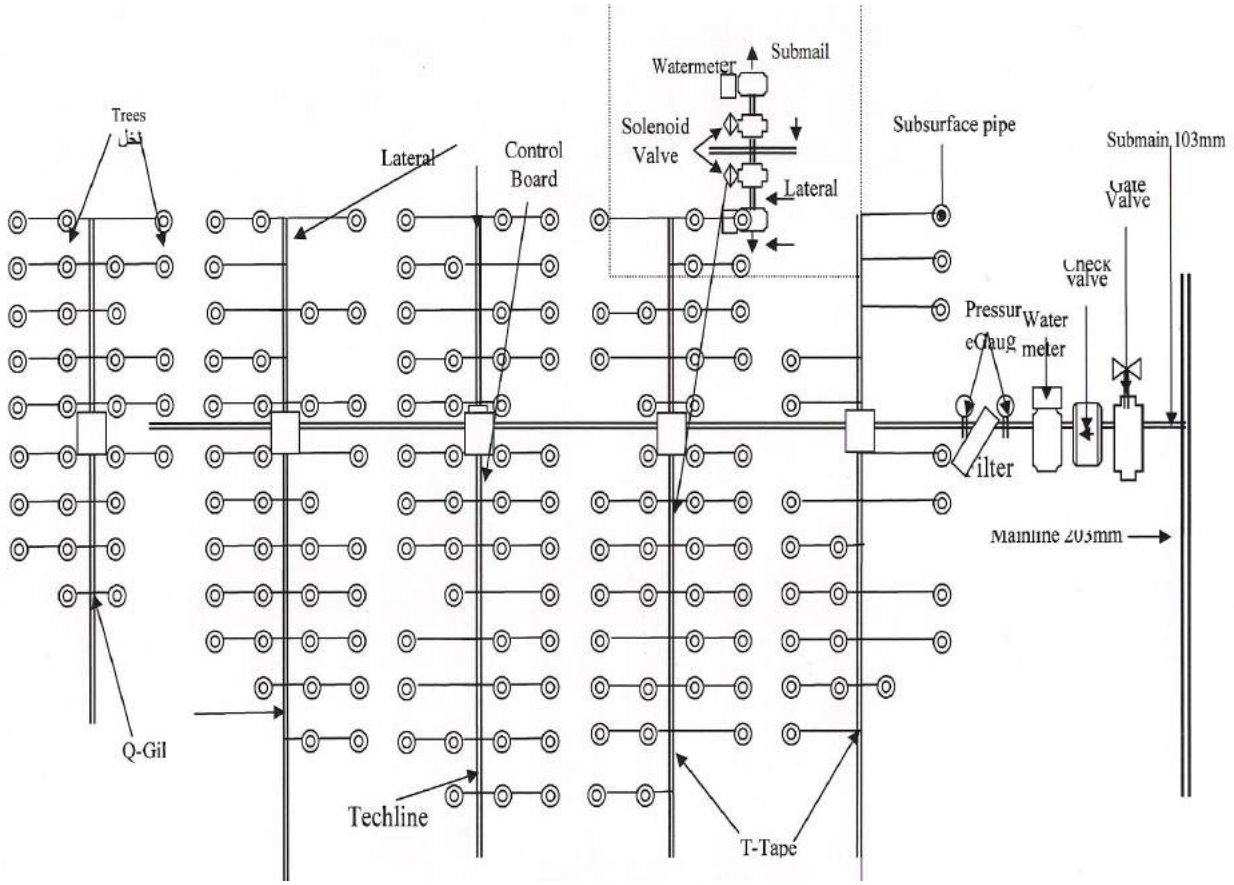


Fig. 2. General design layout.

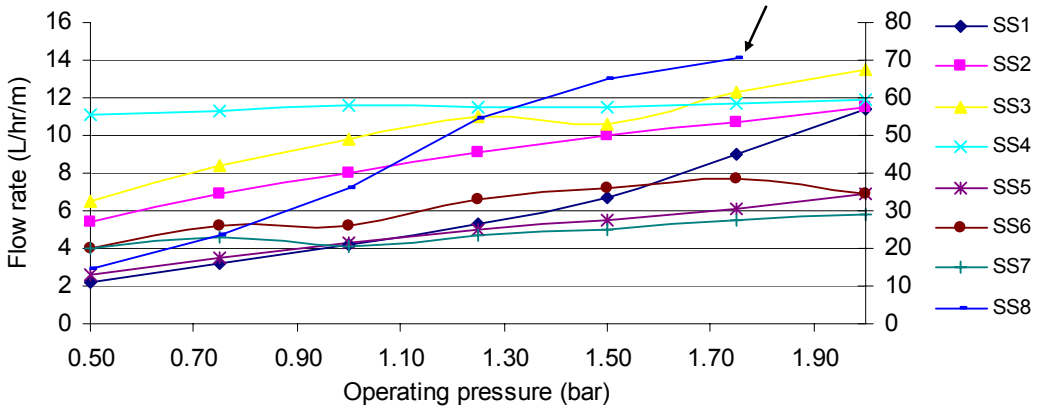


Fig. 3. Curves represent pressure and discharge for subsurface irrigation pipes (the vertical axis of right-special Curve specified arrow).

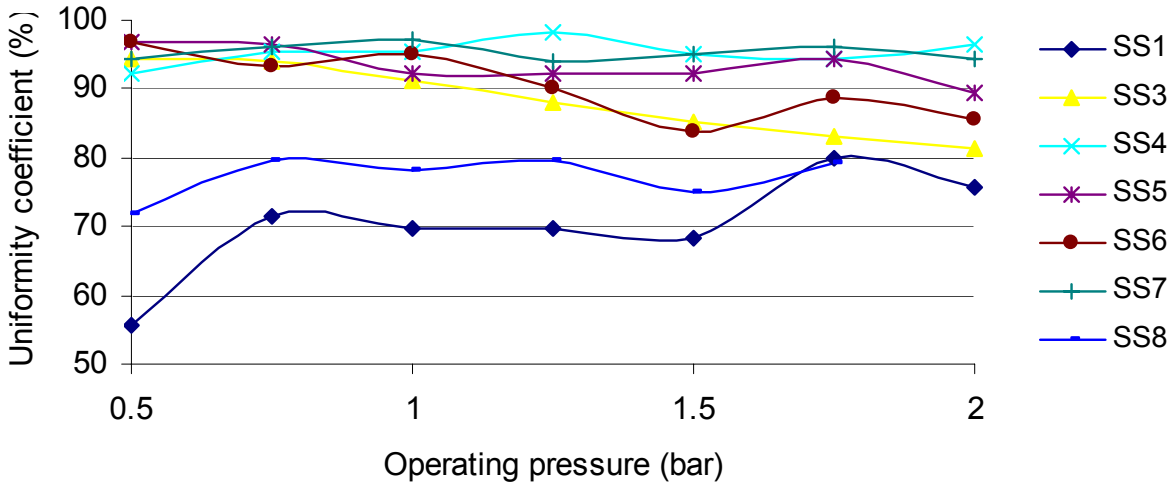


Fig. 4. Uniformity coefficient at various operating pressures for different pipes.

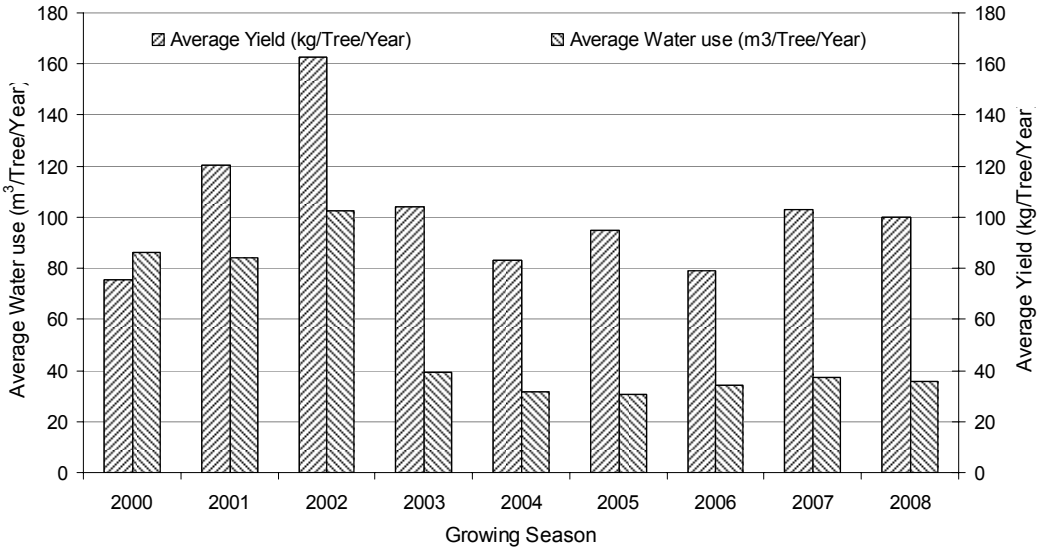


Fig. 5. Date palm water consumption and yield for seasons 2000 to 2008.

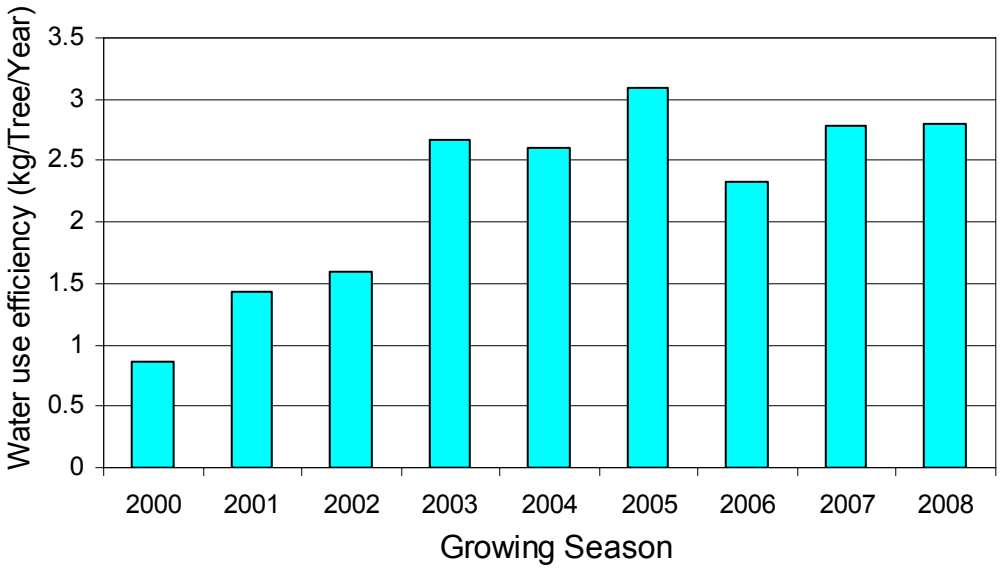


Fig. 6. Water use efficiency for different seasons.

Role of Phosphorus Solubilizing Microorganisms in the Growth of Date Palm Trees

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Keywords: soil phosphorus, solubilisation, organic fertilizers, soil pH, *Bacillus*, mycorrhiza

Abstract

Date palm trees (*Phoenix dactylifera*) tolerate relatively harsh climatic and soil conditions in the UAE and gulf countries. The use of biofertilizers and organic manure to increase the efficiency of phosphorus uptake has been studied. Mycorrhiza fungi and *Bacillus megatherum* bacteria are used separately or in combination to study their role in phosphorus solubilization.

Soil phosphorus is the least mobile element in plants and soil contrary to other macronutrients. A large amount of phosphorus applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils

Soil microorganisms play a key role in soil phosphorus dynamics and subsequent availability of phosphate to plants. Phosphorus solubilizing organisms play a role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil phosphorus pools by solubilization and mineralization.

The principal mechanism in soil for mineral phosphate solubilization is lowering of the soil pH by microbial production of organic acids and mineralization of organic phosphorus by acid phosphatises. Use of phosphorus solubilizing organisms as inoculants increases phosphorus uptake. These microorganisms also increase prospects of using phosphatic rocks in crop production.

Greater efficiency of phosphorus solubilizing bacteria has been shown through co-inoculation with beneficial bacteria and mycorrhiza.

INTRODUCTION

It is true that the date palm tree plays a major role in the life of people in Arabic nations. Date fruits are a key element in food security, therefore scientists pay attention to the care of date palm trees.

The role of fertilizers and their effect on the growth of date palm tree and its yield quantity and quality has been studied and a positive relation between nutrients and tree growth is reported (Badawi and Obaidy, 2009; Shaheen et al., 2003; Patdar and Mali., 2002; Ghaleb and Salim, 2001; Omar, 1997; Salim and Mousa, 1989).

Using biofertilizers could be a natural input for increasing the growth of trees under the severe climate and desert soil. Soil phosphorus is the least mobile element in plants and soil contrary to other macronutrients. A large amount of phosphorus applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (Ahmed et al., 2009).

Inorganic forms of phosphorus are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals and/or chelate cationic partners of phosphorus (He et al., 2002). Phosphate solubilizing microorganism are being used in biofertilizers since 1950 (Kudashev, 1956; Krasilinikov, 1957).

There is strong evidence that soil bacteria are capable of transforming soil

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phosphorus to the forms available to plants. Microbial biomass assimilates soluble phosphorus and prevents it from adsorption or fixation (Khan and Joergesen, 2009). The microbial community influences soil fertility through soil processes viz., decomposition, mineralization, and storage/release of nutrients. Microorganisms enhance the phosphorus availability to plants by mineralizing organic phosphorus in soil and by solubilizing precipitated phosphates (Chen et al., 2006; Kang et al., 2002; Pradhan and Sukla, 2005).

Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe et al., 1998). Phosphate solubilization takes place through various microbial processes/ mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996; Nahas, 1996).

Inorganic P is solubilized by the action of organic and inorganic acids secreted by phosphorus solubilizing microorganisms in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Kpomblekou and Tabatabai, 1994; Stevenson, 2005).

MATERIALS AND METHODS

This study was conducted in the year 2008 at Al-Ain, UAE, to study the effect of biofertilizers on solubization of phosphorus in sandy soils. 25 identical date palm trees of three years old were selected and divided into five groups, each treatment of five replicates.

Treatments were as follows:

1. Control with normally grown trees.
2. Received 25 kg of Al-nawaya organic fertilizer.
3. Received 25 kg of Al-nawaya organic fertilizer + 250 ml of VAM.
4. Received 25 kg of Al-nawaya organic fertilizer + 250 ml of microfert (*Bacillus megatherum*).
5. Received 25 kg of Al-nawaya organic fertilizer + 250 ml of VAM + 250 ml of microfert (*Bacillus megatherum*).

The organic fertilizer contained 2.5% of phosphorus which was derived from rock phosphate and 55% organic matter. All biofertilizers were produced at Emirates Bio Fertilizer factory at AL-Ain, UAE.

Materials were mixed thoroughly around the tree at 15 cm depth and irrigated immediately.

Plant leaves were taken at zero time and after 90 days for the evaluation of nutrients uptake, mainly nitrogen and phosphorus.

Soil samples were taken at the intervals of 0- 30- 60 and 90 days to study the effect of treatments on soil microbial activity, organic acids generated, pH values of the soil and at the end of experiment the level of phosphorus and nitrogen uptake has been reported compared with the initial values in the tree leaves and fronds.

All biological analyses followed the standard methods after APHA (1989). Chemical analyses was done according to Chapman and Pratt (1961).

RESULTS AND DISCUSSION

Table 1 reveals the microbial activities in the soil as affected by the addition of organic matter and inoculation by microorganisms. The relation between microbial activity and soil fertility has been reported by several studies; Alexzander (1977) and Badawi and El-Obaidy (2009).

The addition of organic fertilizer showed enhancement of microbial population throughout the course of the experiment for 90 days. Inoculation of soil by microorganisms increased the population of total plate counts. Interaction between organic matter, bacteria and VAM showed the highest activity of the soil against the control.

Soil phosphorus is the least mobile element in plants and soil contrary to other

macronutrients. A large amount of phosphorus applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (He et al., 2002; Stevensen, 2005; Ahmed et al., 2009).

Table 2 shows the increase of released phosphorus as affected by the addition of biofertilizers and organic matter. It is clear that the interaction between organic matter and VAM and bacteria gave the highest record of available phosphorus.

The principal mechanism in soil for mineral phosphate solubilization is lowering of the soil pH by microbial production of organic acids and mineralization of organic phosphorus by acid phosphatises. The use of phosphorus solubilizing organisms as inoculants increases the phosphorus uptake. These microorganisms also increase prospects of using phosphatic rocks in crop production.

Table 3 reveals the role of organic matter and its microbial activities in releasing organic acids and enzymes which decreased the soil pH value and enhanced the release of phosphorus in the soil solution being more available for uptake by the plants (Evans, 1996; Nahas, 1996).

Tables 4 and 5 summarise the changes in nitrogen and phosphorus in all treatments. From the data presented it is clear that levels of nitrogen and phosphorus increased for all treatments compared with the control. The interaction between VAM and *Bacillus megatherum* showed the highest values for nitrogen and phosphorus content in the tree leaves and fronds. Addition of organic fertilizers enhanced the soil conditions and reflected a positive effect on microbial activities (Badawi and Obaidy, 2009).

The increase of nitrogen and phosphorus contents of plants was due to the actual increase of plant growth. Also mineralization of nutrients in the soil was due to the decrease of soil pH and increase of soil acidity. The activity of microorganisms increased the solubility of phosphorus due to the microbial enzymes produced e.g., acid phosphatise and proteases (Ahmed et al., 2009).

CONCLUSIONS

Since most alkaline soil have high pH values and a high content of lime (calcium carbonate) phosphorus elements are not available for uptake by plants because it is combined with calcium cations and become unavailable to plants. Therefore a great amount of phosphorus could be released to plants by using biofertilizers which is a low cost and environmentally friendly product and will contribute to saving the environment and reduce the application of chemical fertilizers. In the same time we can use rock phosphate instead of synthetic forms.

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Tables

Table 1. Periodical changes of total plate counts (Log numbers) in treated soil.

Treatments	0	30	60	90
Control	6.0	6.1	6.7	6.3
Organic fertilizer	6.6	7.8	8.1	8.0
Organic fertilizer + VAM	6.6	8.2	8.4	8.8
Organic fertilizer + bacteria	6.5	7.8	8.1	8.0
Organic fertilizer + VAM + bacteria	6.8	8.8	8.9	9.1

Table 2. Periodical changes of a available phosphorus (ppm) in the soil during 90 days test.

Treatments	0	30	60	90
Control	130	142	132	144
Organic fertilizer	457	501	533	543
Organic fertilizer + VAM	444	511	583	551
Organic fertilizer + bacteria	339	533	599	611
Organic fertilizer + VAM + bacteria	411	521	574	643

Table 3. Periodical changes of volatile organic acids (ppm) in the soil during 90 days test.

Treatments	0	30	60	90
Control	600	690	711	750
Organic fertilizer	740	2011	2500	2645
Organic fertilizer + VAM	780	2600	2877	3022
Organic fertilizer + bacteria	801	3000	3241	3466
Organic fertilizer + VAM + bacteria	813	3244	3400	3755

Table 4. Levels of nitrogen and phosphorus percentage in leaves and fronds of date palm trees before inoculation by biofertilizers.

Treatments	N in leaves	N in fronds	P in leaves	P in fronds
Control	0.66	0.51	0.055	0.036
Organic fertilizer	0.69	0.55	0.088	0.043
Organic fertilizer + VAM	0.71	0.52	0.095	0.061
Organic fertilizer + bacteria	0.66	0.57	0.099	0.065
Organic fertilizer + VAM + bacteria	0.77	0.66	0.120	0.067

Table 5. Levels of nitrogen (N) and phosphorus (P) percentage in leaves and fronds of date palm trees as affected by inoculation by biofertilizers.

Treatments	N in leaves	N in fronds	P in leaves	P in fronds
Control	0.95	0.82	0.11	0.066
Organic fertilizer	1.32	0.87	0.13	0.073
Organic fertilizer + VAM	1.44	0.94	0.14	0.081
Organic fertilizer + bacteria	1.80	1.25	0.16	0.095
Organic fertilizer + VAM + bacteria	1.95	1.32	0.166	0.097

The Role of NAA in the Regulation of Mineral Concentration of Date Palm Seedlings ('Shabeeby') under Salt Stress Conditions

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Keywords: *Phoenix dactylifera*, irrigation, salt water, NAA, leaves, roots, concentrations, NaCl

Abstract

In many countries including Qatar, salinity in irrigation water is a serious problem for agriculture. Irrigation with saline water reduces the plant growth and productivity of most fruit trees. An experiment was carried out at the Research Station of Qatar University, to study the effect of NAA on mineral concentrations of date palm (*Phoenix dactylifera* L. 'Shabeeby') seedlings. The effects of NAA alone (0, 150 , 300 mg/L) or in combination with NaCl and NaCl alone (0, 6000, 12000, 18000 mg/L) added to the irrigation water were studied. After 115 days from the first treatment, concentrations of N, Ca, Mg, Cu, Mn, Zn in leaves and N, P, K, Ca, Mg, Cl, Cu, Mn, Zn and Fe in roots significantly increased with the application of high NAA in 'Shabeeby' date palm seedlings.

High salinity concentrations in irrigation water decreased leaf P, Cu, Mn, Zn and Fe but increased K, Na and root Cl and Cu concentrations relative to the control seedlings. The combination of high NAA with high salinity in irrigation water reduced the adverse effects of salt, preventing the reduction of most mineral elements in leaves and increased the concentrations of others in roots. However, the increases of Na in leaves and Na, Cl, N, P, Ca and Mg in roots with the application of high NaCl in combination with high NAA concentrations in irrigation water, without any symptoms of NaCl stressed indicated that the high accumulation of Na, Cl and other elements might have played an important role in osmotic adjustment in vacuoles and that it improves the water balance of 'Shabeeby' seedlings. Compared to high NaCl concentration used alone, the irrigation of date palm seedlings with a high concentration of NAA in combination with salts added to the water reduced the adverse effects of salt by preventing the reduction of mineral elements concentration in leaves and increased the element concentrations in roots.

INTRODUCTION

*S*alinity in irrigation water is a serious problem for agriculture (Grattan, 2002). Irrigation with saline water has been found to depress plant growth and productivity of date palm (Al-Dabbas, 2003; Aljuburi and Al-Masry, 2000; Hassan and El-Samnoudi, 1993; Sane et al., 2005). It was reported that the irrigation of date palm seedlings, with saline water in combination with naphthalene acetic acid (NAA) indole acetic acid (IAA) and gibberellic acid (GA_3) significantly increased the shoot dry matter percentage (Aljuburi and Al-Masry, 1996; Aljuburi et al., 2002).

Leaf Na, Cl, iron (Fe) concentrations of date palm seedlings, increased when irrigated with salt application alone or in combination with GA_3 (Aljuburi, 1996; Aljuburi

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and Al-Masry, 2000) or IAA (Aljuburi and Al-Masry, 2000). Similarly, the application of salt in irrigation water increased, Cl, K, Na, nitrogen (N) and phosphorus (P) concentrations, but reduced calcium (Ca) and Magnesium (Mg) concentrations in the shoots of most citrus rootstock (Al-Jabery, 1993; Zekri, 1993). Moreover, according to other results (Aljuburi and Al-Masry, 2000; Zid and Grignon, 1987) the application of saline water had no significant effect on N, P, K concentrations, but led to large Cl and Na accumulation in *Citrus aurantium* leaves. The accumulation of high Na and Cl concentrations in date palm leaves may provide a beneficial physiological activity through the osmotic adjustment (Hassan and El-Samnoudi, 1993).

Application of GA_3 alone or in combination with salt in irrigation water on date palm seedlings significantly increased the shoot and root Na and Cl concentration (Aljuburi, 1996; Aljuburi and Al-Masry, 2000).

The objective of this research was to investigate the potential improvement in growth and the increase of ion content of 'Shabeeby' date palm seedlings under salt stress conditions due to the addition of NAA.

MATERIAL AND METHODS

The experiment was conducted in the nursery of the Experimental Station of Qatar University at Rawdat Al-Faras region in Qatar State. One-year-old, uniform date palm (*Phoenix dactylifera* L.*)* seedlings of the 'Shabeeby' cultivar were transplanted in 40 cm in diameter and 30 cm deep plastic bags filled with peatmoss and yellow sand. All plants were irrigated with tap water three times a week for 115 days. Plants were arranged in a completely randomized block design, with salt concentrations 0, 6000, 12000 and 18000 mg/L. NaCl treatments were imposed by irrigating each plant once a week with 300 ml of the corresponding salt solution in addition to 300 ml of various NAA solutions (150 and 300 mg/L) sprayed monthly. The experiment consisted of 12 treatments, replicated 3 times, with one seedling as the experimental unit.

The nitrogen content of leaves and roots was determined by the micro-Kjeldahl method. Measurement of Ca, Fe, K, Mn, Mg, Na, Zn and Cu concentrations was conducted after wet digestion with a mixture (4:1) of nitric/perchloric acid by atomic absorption spectrophotometer (GBC Avanta). The phosphorus concentration was determined by colorimeter method "spectrophotometer" (UNICAM 8620 UV/V1S spectrometer). The chloride concentration was measured using the Ion-chromatograph (DIONEX IC 25) technique (Walinga et al., 1989; Westerman et al., 1990).

The data were subjected to analysis of variance and LSD was used for mean comparison with $P<0.05$.

RESULTS

Salinity and NAA Effects on Leaf Mineral Concentrations

Irrigation of 'Shabeeby' seedlings with low salt level concentration (6000 mg/L) significantly decreased leaf N concentration as compared with the check treatment (Table 1). The leaf N concentration of 'Shabeeby' seedlings significantly increased, when sprayed with 300 mg/L NAA alone as compared with the non-treated plants. The results of the salinity effect on the leaf N level are in agreement with other researchers (Aljuburi, 1996; Aljuburi and Al-Masry, 1996, 2000) who showed that salinity in irrigation water reduced the N concentration of date palm leaves. The results of the NAA effect on the leaf N concentration of 'Shabeeby' seedlings are in agreement with those of Hale and Orcutt (1987), who reported that the plant growth regulators application resulted in higher concentrations of N in the stem tissues of tomato plants.

Application of a low concentration of NAA (150 mg/L) or salinity (6000, 12000 or 18000 mg/L) alone or a combination with 300 mg/L NAA, with 6000 mg/L NaCl or 150 mg/L NAA with 1200 mg/L NaCl reduced the seedling leaf P concentration as compared with the control (Table 1). The results of the leaf P concentration of 'Shabeeby' seedlings are in agreement with those of other studies (Aljuburi, 1996), which showed

that salinity in irrigation water decreased the leaf P concentration in date palm.

The leaf K concentration of 'Shabeeby' seedlings significantly decreased when irrigated with a low concentration of saline water as compared with the control (Table 1). However, application of a high NaCl concentration alone or with low NAA to 'Shabeeby' seedlings increased the leaf K concentration over the control. These findings are in agreement with Aljuburi (1996) who showed that application of a low GA_3 concentration to 'Lulu' date palm seedlings increased the shoot K concentration over the control, and with those of Aljuburi and Al-Masry (1996) and Nawar and Ibrahim (1984) who showed that salinity significantly increased the K concentration in citrus and pear leaves and roots.

Irrigating 'Shabeeby' date palm seedlings with the NAA solution alone or in combination with medium or high salinity in irrigation water or low NAA with low NaCl concentrations significantly increased leaf Ca concentration respectively as compared with the control (Table 1). Irrigation with water containing salts (6000 or 12000 mg/L) significantly decreased leaf Ca concentrations as compared with the control.

Application of high concentrations of NAA or a medium concentration of NaCl on 'Shabeeby' date palm seedlings significantly increased the leaf Mg concentration, as compared with the control (Table 1). The results of NAA are in agreement with those of Hale and Orcutt (1987) who reported that the application of plant growth regulators on tomato plants increased the stem Mg concentrations as compared with the control.

The leaf Na concentrations of 'Shabeeby' seedlings were significantly increased when irrigated with a high concentration of saline water alone or in combination with a high NAA concentration or a low NAA concentration in combination with medium salt concentration relative to the non-treated seedlings (Table 1). Application of NAA alone or in combination with low salinity in irrigation water had no significant effect on Na level of 'Shabeeby' seedling leaves, whereas addition to irrigation water of 300 mg/L NAA in combination with 12000 mg/L NaCl, or 150 mg/L NAA in combination with 12000 mg/L Nacl, 150 mg/L NAA in combination with 18000 mg/L NaCl did not significantly change leaf Na concentrations of treated seedlings as compared with the non-treated seedlings.

These results are in agreement with others (Aljuburi, 1996; Aljuburi and Al-Masry, 2000), which demonstrated that salinity in irrigation water increased leaf, Na concentrations in date palm. Also they showed that the application of indole acetic acid (IAA) alone or in combination with salinity or GA_3 (gibberellic acid) in combination with salinity in irrigation water on date palm seedlings, 'Lulu' or 'Khalas' and 'Lulu', increased the leaf Na concentration.

The leaf Cl concentration of 'Shabeeby' seedlings was not significantly affected by application of NAA alone or in combination with saline irrigation water as compared with the control. The results of the NAA effect on the leaf Cl concentration of 'Shabeeby' seedlings are in agreement with those of Aljuburi (1996), showing that gibberellic acid had no effect on 'Khalas' and 'Lulu' leaf Cl concentration.

Application of NAA on 'Shabeeby' seedlings significantly increased the leaf Cu concentration as compared with the control. The leaf Cu levels of 'Shabeeby' seedlings also significantly increased when irrigated with a combination of 300 mg/L NAA and low and medium NaCl concentrations relative to the control (Table 1).

Irrigation of 'Shabeeby' seedlings with a high salt concentration alone, significantly decreased the leaf Cu levels relative to non-treated seedlings.

The results of plant growth regulators on leaf Cu concentrations are in agreement with others (Hale and Orcutt, 1987) which demonstrated that application of plant growth regulators resulted in higher leaf mineral concentrations in the stem tissues of tomato plants. Moreover, the application of saline water to citrus plants decreased Cu concentrations in leaves and roots (Atalla, 1987; Banuls et al., 1990).

Leaf Mn concentrations of 'Shabeeby' seedlings were significantly increased when irrigated with NAA alone relative to the non-treated seedlings. Application of medium or high salt alone significantly decreased leaf Mn levels as compared with the control.

Application of a high NAA concentration in combination with medium and high salinity in irrigation water significantly increased leaf Mn levels of 'Shabeeby' seedlings as compared with the application of medium and high salt concentrations alone (Table 1). These results agreed with previous work of Aljuburi (1996), which showed that date palm seedling irrigation with salt alone significantly decreased leaf Mn concentrations in the 'Khalas' seedlings. Similar results of growth regulators effects on leaf Mn concentrations of 'Lulu' seedlings and tomato plants were also obtained in previous studies (Aljuburi, 1996; Hale and Orcutt, 1987).

Irrigation of 'Shabeeby' seedlings with saline water alone significantly reduced the leaf Zn whereas application of a high concentration of NAA alone significantly increased the leaf Zn as compared with non-treated seedlings (Table 1).

Leaf Zn concentrations of 'Shabeeby' seedlings significantly increased when irrigated with high concentrations of NAA in combination with low and medium levels of NaCl concentrations as compared with the application of salt alone. These findings are also in agreement with those of (Aljuburi, 1996; Banuls et al., 1990) who showed that the salinity decreased shoot Zn concentrations of 'Khalas' and 'Lulu' and citrus plants .

Other workers (Hale and Orcutt, 1987) demonstrated that the plant growth regulator led to accumulation of Zn in tomato plants.

When irrigating 'Shabeeby' seedlings with high concentrations of the NAA solution alone or high, medium or low salt concentrations alone in irrigation water the leaf Fe levels of 'Shabeeby' seedlings significantly decreased compared with the control (Table 1).

High NAA in combination with low salt concentrations decreased leaf Fe concentrations relative to the control. The results of the salinity effect on leaf Fe level of 'Shabeeby' seedlings are in agreement with those of Banuls et al. (1990) who found that the application of saline water to citrus plants decreased Fe concentrations in leaves and roots.

Salinity and NAA Effects on Root Mineral Concentration

Root N or P concentrations in 'Shabeeby' seedlings significantly increased with application of NAA in irrigation water alone or low or high concentration in combination with low or high salinity in irrigation water respectively as compared with the control seedlings (Table 2). Application of GA_3 or IAA alone or in combination with saline water increased root N or P concentrations of 'Khalas' or 'Lulu' seedlings respectively (Aljuburi, 1996; Aljuburi and Al-Masry, 2000).

Low NAA in combination with low NaCl concentrations, significantly increased the root K concentrations of 'Shabeeby' seedlings over the control (Table 2). As compared with the control seedlings, root K concentrations of 'Shabeeby' seedlings significantly increased, when irrigated with NAA alone.

High NAA in combination with high salt concentrations or high NAA in combination with medium NaCl concentrations significantly increased the root Ca, Mg or Mg concentrations respectively over the control. Application of NAA alone significantly increased root Ca and Mg concentration over the control. The results of the plant growth regulators effect on root Ca, Mg concentrations of 'Shabeeby' seedlings are in agreement with those of Hale and Orcutt (1987), who demonstrated that the application of plant growth regulators resulted in higher concentrations of Ca, Mg in the stem tissues of tomato plants.

Root Na concentrations of 'Shabeeby' seedlings significantly increased when irrigated with low concentrations of NAA application alone or in combination with medium salt concentrations, or high NAA concentrations in combination with high salt concentrations relative to the non-treated seedlings. High NAA concentrations or salts alone, or the combination of NAA with low salinity concentrations in irrigation water, high NAA with medium salt or low NAA concentrations with high salinity concentrations in irrigation water had no significant effect as compared to non-treated seedlings. The results of root Na concentrations of 'Shabeeby' seedlings agree with those of Aljuburi

(1996) and Aljuburi and Al-Masry (1996, 2000) who showed that the salinity in irrigation water in combination with plant growth regulators increased root Na concentrations of 'Khalas' and 'Lulu' or 'Lulu' date palm seedlings, the results of the salinity effect on root Na concentrations of 'Shabeeby' seedlings are in agreement with those of Aljuburi and Al-Masry (1996), who showed that the salinity had no effect on leaf Na concentrations of five citrus rootstock seedlings as compared with the control.

The results also showed that the root Na concentrations were higher than leaf Na concentrations for most treatments. These results agree with those previously obtained with the use of IAA and salinity to irrigate 'Lulu' seedlings (Aljuburi and Al-Masry, 2000).

Root Cl concentrations in 'Shabeeby' seedlings significantly increased with application of high salinity in irrigation water alone or in combination with high NAA concentrations over the control. However, NAA application alone or in combination with medium concentration of salts in irrigation water, significantly increased root Cl concentrations of 'Shabeeby' seedlings over the control (Table 2). The results of the effect of high salinity concentrations in irrigation water on root the Na concentration of 'Shabeeby' seedlings are in agreement with other works (Aljuburi, 1996; Hassan and El-Samnoudi, 1993; Zekri, 1993), which showed that the salinity led to accumulation of Cl and Na in date palm and citrus seedlings, respectively. The results of plant growth regulator in combination with salinity in irrigation water are in agreement with those of Aljuburi (1996) who showed that the salinity in combination with GA_3 increased root Na and Cl concentrations of 'Lulu' and 'Khalas' date palm seedlings. These results also showed that the root Cl concentrations were lower than leaf Cl concentrations whereas the root Na concentrations were higher than leaf Na concentrations.

High NAA concentrations significantly increased the root Mn concentration over the control. Similar results have already been obtained (Aljuburi and Al-Masry, 2000) with 'Lulu' seedlings.

Application of NAA alone or low salt concentrations alone in irrigation water significantly increased root Zn concentrations of 'Shabeeby' seedlings as compared with the control (Table 2). High NAA concentrations alone, added in the irrigation water, significantly increased the root Fe concentrations as compared with the control.

The application of IAA significantly increased Zn concentrations and decreases the Fe concentrations in roots.

DISCUSSION

The results revealed that the application of low NAA concentrations on 'Shabeeby' seedlings significantly increased leaf Ca, Cu, Mn or root N, P, K, Ca, Mg, Na, Cl, Cu and Zn and significantly decreased leaf P concentrations as compared with the control. Leaf N, Ca, Mg, Cu, Mn, Zn or root N, P, K, Ca, Mg, Cl, Cu, Mn, Zn and Fe concentrations significantly increased, but leaf K and Fe concentrations decreased with application of high NAA concentrations on 'Shabeeby' date palm seedlings. Leaf Na concentrations significantly increased with irrigation of 'Shabeeby' seedlings with high concentration of saline water alone or in combination with high NAA, or medium salinity with low NAA concentrations as compared with the control, whereas root Na concentrations significantly increased with application of low NAA concentration alone or in combination with medium levels of NaCl or high NAA with high salinity in irrigation water relative to the control.

Application of salts or NAA in irrigation water alone or in the combination had no significant effect on 'Shabeeby' leaf Cl concentrations, whereas root Cl concentrations significantly increased with the application of NAA or high salinity concentrations in irrigation water alone or NAA in combination with medium concentrations of salt, or high NAA with high NaCl concentrations in irrigation water as compared with the control.

The result could support the hypothesis, which showed that the application of NAA, could be assisted of accumulating the mineral elements in leaf and root of treated seedlings, therefore, could increase the plant growth, then plant resistance to salt stress is

increased or when exogenous NAA is sprayed on 'Shabeeby' seedlings, NAA might be bound to receptors and activated the ATP-driver pump. As hydrogen ions (H^+) are being pumped out the cell, the cell wall becomes acidic, breaking hydrogen bonds. Cellulose fibrils are weakened and activated enzymes further degrade the cell wall. More water enters the cell, and therefore turgor pressure increases. The resulting increase in turgor pressure causes the cell to expand and further stimulates the loosening of bonds between cellulose micro-fibrils. Expansion of the micro-fibrils results in elongation of the cell and dilutes the concentration of Na and Cl or binding them with other compounds or translucated Na and Cl to vacuole (Marder, 2004).

Salinity significantly reduced or had no effect on element concentrations of leaf and root. The increases of Na in leaf or Na and Cl in roots with application of high or medium salinity concentrations in irrigation water without any symptoms of NaCl stresses could indicate that the high accumulation of Na and Cl could play an important role as osmotic adjustment in vacuoles and that it improves the water balance. Aljuburi (1996) obtained similar results and Hassan and El-Samnoudi (1993), who reported that the absorption of Na and Cl by plants serves a useful function by providing lower osmotic solutes.

Application of high NaCl concentration alone increased the leaf K, Na, root Cl, and Cu and decreased the leaf P, Cu, Mn, Zn, and Fe concentrations, whereas the combination of high NAA and high NaCl concentrations in irrigation water increased leaf Na levels only and root N, P, Ca, Mg, Na and Cl concentrations, as compared with the control. Therefore these results could be indicate that spraying NAA on salt stressed seedlings increased plant resistance by increasing the concentrations of the mineral elements in roots.

These findings are in agreement with other workers (Hale and Orcutt, 1987) who showed that plant growth regulators could improve the nutrient uptake and the efficiency of use, by altering the rate of membrane selectivity of elements uptake, and improve root growth and thus increase the volume of soil penetrated by roots.

CONCLUSIONS

Compared to saline water used alone, the irrigation of date palm seedlings with naphthalene acetic acid in combination with salt added to irrigation water reduced the adverse effect of salinity by preventing the reduction of most mineral elements in leaves and increased the most macro elements in root. In addition, from the results could be concluded that the application of 300 mg/L of NAA alone or in combination with a high NaCl concentration in irrigation water increased most of the leaves and roots elements or roots element respectively. This accumulation of elements could increase the biochemical reactions and act as adjust osmotic regulation of seedlings, and finally the growth rate increased. Therefore, these treatments could be recommended for the Qatar region.

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Tabels

Table 1. Effect of different levels of NAA and saline water on leaf nutrient concentrations of date palm (*Phoenix dactylifera* L. 'Shabeeby') seedlings*.

Treatment			N	P	K	Ca	Mg	Na	Cl	Cu	Mn	Zn	Fe
			Dry weight (%)								Dry weight (ppm)		
Control			1.16	0.120	1.13	0.583	0.287	0.260	1.273	3.12	40.11	28.81	195.52
150 mg NAA/L			1.30	0.090	1.06	0.790	0.303	0.293	1.522	5.86	53.63	25.38	169.09
300 mg NAA/L			1.36	0.113	0.98	1.050	0.507	0.327	1.439	5.43	51.37	58.65	131.33
6000 mg NaCl/L			0.85	0.050	0.88	0.423	0.220	0.377	1.024	2.77	38.80	18.67	129.31
12000 mg NaCl/L			1.09	0.083	1.12	0.457	0.200	0.230	1.156	3.07	29.94	16.41	127.71
18000 mg NaCl/L			1.09	0.083	1.40	0.593	0.263	0.520	1.436	1.77	26.24	19.84	126.26
150 mg NAA/L + 6000 mg NaCl/L			1.29	0.093	1.13	0.713	0.310	0.500	1.441	4.42	41.07	27.61	153.43
300 mg NAA/L + 6000 mg NaCl/L			0.91	0.050	1.05	0.703	0.253	0.440	1.560	5.31	44.61	34.54	130.19
150 mg NAA/L + 12000 mg NaCl/L			0.97	0.057	0.98	0.677	0.283	0.633	1.531	3.84	33.53	21.68	165.32
300 mg NAA/L + 12000 mg NaCl/L			1.39	0.093	1.30	0.830	0.383	0.533	1.606	5.25	45.82	36.22	162.74
150 mg NAA/L + 18000 mg NaCl/L			1.46	0.127	1.49	0.797	0.313	0.470	1.492	4.45	29.60	27.31	156.45
300 mg NAA/L + 18000 mg NaCl/L			1.17	0.100	1.18	0.697	0.360	0.617	1.771	3.23	43.99	25.01	175.96
L.S.D.	0.05	NaCl	0.18	0.035	0.17	0.074	0.074	0.182	0.301	0.83	8.65	8.68	34.64
		NAA	0.16	0.030	0.15	0.064	0.064	0.158	0.260	0.72	7.49	7.51	30.00
		NAA × NaCl	0.32	0.060	0.29	0.128	0.128	0.316	0.521	1.44	14.98	15.03	60.00
	0.01	NaCl	0.25	0.047	0.23	0.100	0.100	0.248	0.409	1.32	11.76	11.79	47.08
		NAA	0.21	0.041	0.19	0.087	0.087	0.215	0.354	0.98	10.18	10.21	40.78
		NAA × NaCl	0.43	0.081	0.39	0.174	0.174	0.429	0.708	1.96	20.36	20.43	81.55

* values are mean of three seedlings: three replications each with one seedlings.

Table 2. Effect of different levels of NAA and saline water on root nutrient concentrations of date palm (*Phoenix dactylifera* L. 'Shabeeby') seedlings*.

Treatment			N	P	K	Ca	Mg	Na	Cl	Cu	Mn	Zn	Fe
			Dry weight (%)							Dry weight (ppm)			
Control			0.34	0.023	1.01	0.317	0.350	0.660	1.732	1.08	12.51	10.46	198.74
150 mg/NAA/L			0.69	0.050	1.68	0.600	0.950	1.150	2.661	3.85	13.33	15.36	300.29
300 mg NAA/L			0.95	0.097	1.43	0.617	0.623	0.673	2.633	4.12	19.80	17.76	414.46
6000 mg Nacl/L			0.40	0.020	0.88	0.307	0.377	0.737	1.900	2.27	15.95	14.65	213.12
12000 mg NaCl/L			0.34	0.030	0.93	0.397	0.420	0.637	2.411	2.52	14.41	9.78	226.86
18000 mg NaCl/L			0.36	0.030	1.03	0.383	0.397	0.823	3.120	3.68	14.58	10.82	260.43
150 mg NAA/L + 6000 mg NaCl/L			0.61	0.057	1.44	0.423	0.520	1.000	2.640	3.38	12.12	11.22	280.79
300 mg NAA/L + 6000 mg NaCl/L			0.39	0.033	1.30	0.470	0.487	0.943	2.614	3.43	18.67	12.85	366.57
150 mg NAA/L + 12000 mg NaCl/L			0.43	0.033	1.29	0.450	0.480	1.777	3.079	4.32	13.98	11.06	258.96
300 mg NAA/L + 12000 mg NaCl/L			0.45	0.043	1.27	0.473	0.577	1.063	3.676	3.57	14.22	11.24	314.05
150 mg NAA/L + 18000 mg NaCl/L			0.50	0.040	0.98	0.387	0.480	1.043	2.783	2.80	16.13	11.21	267.48
300 mg NAA/L + 18000 mg NaCl/L			0.71	0.050	1.26	0.590	0.647	1.297	3.716	2.47	20.39	11.87″	281.27
L.S.D.	0.05	NaCl	0.11	0.015	0.24	0.095	0.104	0.252	0.754	1.42	5.13	3.01	126.31
		NAA	0.10	0.013	0.21	0.082	0.090	0.218	0.653	1.23	4.44	2.61	109.38
		NAA × NaCl	0.19	0.026	0.42	0.164	0.180	0.437	1.307	2.45	8.88	5.22	218.77
	0.01	NaCl	0.15	0.021	0.33	0.129	0.141	0.343	1.025	1.92	6.97	4.09	171.68
		NAA	0.13	0.018	0.29	0.112	0.122	0.297	0.88	1.67	6.03	3.55	148.68
		NAA × NaCl	0.27	0.036	0.58	0.223	0.245	0.594	1.776	3.33	12.07	7.09	297.35

* values are mean of three seedlings: three replications each with one seedlings.

Yield and Yield Components of 'Sayer' Date Palm as Affected by Levels and Methods of Iron Fertilization

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Keywords: Fe injection, date palm, fertilization

Abstract

Date palm is one of the most important and strategic agricultural crops. Date palm is cultivated in calcareous soils of Iran. Plants grown on calcareous soils sometimes suffer from an iron deficiency. It has been suggested that inorganic iron compounds, iron chelates, organic compounds, industrial by-products and wastes, acidifying soil amendments and injection, could affect correction. Due to the importance of date palm and so the importance of iron in date palm nutrition this research was conducted in order to study the effects of iron fertilization on yield and yield components of date palm 'Sayer', on 54 trees in the Ahwaz research station of Khoozestan Province on eight-year-old date palm (*Phoenix dactylifera*) in Iran during 2002-2006. Research treatments consisted of: 1) Control treatment, 2) Soil surface application of Fe in two levels, 3) Application of Fe as localized placement method in two levels, 4) Fe injection into the trunk of tree in four levels. The experiment was performed as a randomized complete blocks design with nine treatments and three replications. Agro-technical practices were done according to the custom of the region. Production was harvested and weighted each year at the end of September. Yield, yield components, chemical and physical analyses of leaves and fruits were also done. Data were analyzed statistically and means were compared with Duncan's multiple range tests using MSTATC software. Results showed that in most cases, injection of 25 g $FeSO_4$ tree^{-1} into the trunk showed the best results. Other injection treatments were placed in second class.

INTRODUCTION

The whole land under date cultivation in Iran is estimated at about 240,000 ha (FAO, 2007) of which over 37,000 ha are allotted to Khuzestan province (Anonymous, 2006). 'Sayer' is the most commercial date cultivar in Khoozestan province. The area under cultivation of this cultivar has regularly increased in recent years because of its desirable taste, size and moisture and its important role in export.

Fruit trees and among them date palm, need optimum amounts of minerals for their best growth. Proper application of macro- and micro-nutrient fertilizers is necessary to increase quantitative, qualitative and economical output of date production in palm groves. Fe deficiency or less mobility of Fe in plants prevents chlorophyll formation and causes chlorosis. Fe deficiency can also cause a decrease in assimilation and decline in yield (Malakouti and Tabatabaei, 1999).

A research done on the date palm showed that Fe injection into the trunk of the tree caused a yield increase (Abo-Rady et al., 1987). It was also reported that injection of Fe into the trunk of date palm caused a meaningful increase in Fe concentration in leaves, as well as date yield (Abo-Rady et al., 1987; Rasouli and Malakouti, 1999). Other researchers have also showed that Fe deficiency was removed in some trees such as olive and peach after Fe injection into their trunks (Fernandez et al., 1993). Peryea and Kammereck (1997) observed that trunk injection of iron could eliminate leaf chlorosis in iron-deficient pear trees.

Desirable effects of using iron chelate on chlorosis removal have also been reported in citrus trees (Banuls et al., 2003).

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A research done on the date palm with treatments of 25, 50 and 100 g $FeSO_4$ $tree^{-1}$ showed that injection of 25 g $FeSO_4$ $tree^{-1}$ into the trunk caused the best results. Other injection treatments were placed in second class (Saleh, 2008).

Regarding the mentioned research, it is suggested that there is a significant relation between iron fertilization and yield and chemical composition of date palm in a way that using optimum amounts of Fe fertilizers causes an increase in yield and develops fruit quality and chemical composition of leaves. The objective of this study was to examine the above mentioned hypothesis on 'Sayer' date palm in Khoozestan province.

For this purpose, we studied the effects of using different methods and levels of iron fertilizations on date yield and some other plant responses such as fruit weight average, fruit length, Fe and Zn content of leaves and total sugar percentage of fruits.

MATERIALS AND METHODS

The present research was conducted on 54 date palms 'Sayer' in the Ahwaz research station during 4 years. The experiment was performed on 8-year-old trees as a randomized complete blocks design consisting of 9 treatments and 3 replications with two trees in each treatment.

Research treatments including three fertilization methods in various levels were applied as follows:

1. Control treatment.
2. Soil surface application of 100 g Fe-EDDHA $tree^{-1}$ in the form of a strip around the trunk of trees.
3. Soil surface application of 200 g Fe-EDDHA $tree^{-1}$ in the form of a strip around the trunk of trees.
4. Localized placement of 1 kg $FeSO_4$ $tree^{-1}$.
5. Localized placement of 2 kg $FeSO_4$ $tree^{-1}$.
6. Injection of 2 L solution with zero concentration of Fe and a pH of 3.5.
7. Injection of 25 g $FeSO_4$ $tree^{-1}$ as 2 L solution with a pH of 3.5.
8. Injection of 50 g $FeSO_4$ $tree^{-1}$ as 2 L solution with a pH of 3.5.
9. Injection of 100 g $FeSO_4$ $tree^{-1}$ as 2 L solution with a pH of 3.5.

Besides, the following necessary fertilizers were uniformly used for each tree: 1.5 kg ammonium sulfate, 0.750 kg ammonium phosphate, 1 kg powdery sulfur fertilizer and necessary amounts of animal manure for filling pits. Meanwhile, this fertilizer was added to the soil in 3 pits around the trees with 60 cm in depth and 40 cm in diameter. Agro-technical practices (such as pollination, thinning, irrigation and so on) were done according to the custom of the region. Production was harvested and weighted each year at the end of September. Physical and chemical properties of fruit such as length, average weight, weight ratio of fruit pulp to its stone and fruit total sugar percentage were measured in the laboratory. Also, concentrations of Fe and Zn in leaves were determined. Data were statistically analyzed. Means were compared using Duncan's multiple range tests via MSTATC software.

RESULTS AND DISCUSSION

Injection of 25 g $FeSO_4$ $tree^{-1}$ (treatment 7) caused the highest yield (Table 1). So, in calcareous soils like soils in Khoozestan province, there is a low amount of plant available Fe, despite the large amounts of total Fe in them because of the effect of high pH on reducing Fe availability and so, iron uptake by plant becomes low.

Since the injection of iron into the trunk conveys this element directly to the respective parts of plant, using this method could help us to resolve the problem of absorption and transmission of Fe in date palm. The presence of sufficient amounts of available Fe causes an increase in photosynthesis and carbohydrate motion in plants. This makes more production yield (Mengel and Kirkby, 1978). Yield increase with Fe injection into the trunk of trees was consistent with findings of others (Abo-Rady et al., 1987; Peryea and Kammereck, 1997). There are some other effective ways to do this (Tindall et al., 1996; Malakouti and Samar, 1998; Malakouti and Tabatabaei, 1999).

There were no statistical differences between fruit weights in treatments. However, from the numeral viewpoint the highest and lowest amounts were seen in treatments 7 and 1, 6, respectively of which treatment 1 and 6 are the control treatment and a treatment without Fe applied. Anyway, injection of 25 g $FeSO_4$ $tree^{-1}$ and other applied iron treatments had the best effects. Similar results were reported by Saleh (2008). It can be explained with the effect of Fe on increasing the plant Fe concentration that consequently enhances the photosynthesis rate in the plant (Mengel and Kirkby, 1978). Increasing the fruit average weight due to iron injection into the trunk of trees has also been reported by others (Rasouli and Malakouti, 1999).

Fruit length was the same in all of the treatments, statistically. But, treatment 7 showed the highest fruit diameter, that is 18.21 mm and was significant different with the other treatments. The lowest diameter of the fruit was measured in treatment 1 and 6, that is, the control treatment and the treatment without Fe applied. Similar results were reported by Moghimi (1998).

Characteristics such as weight of fruit and brunch, percent of fruit set, fruit diameter, stone diameter, spathe width, trunk circumference, length of thorn, leaflet and leave, Fe (leave), Zn (leave) increased as effect of treatment 7.

Fe injection increased the average of fruit weight, via injection into the trunk of the tree. The highest amounts of Fe concentration in date leaves were seen in treatments 7 and 9. Treatment 4 showed a lower amount of leaf Fe concentration, statistically. Similar results were reported by Saleh (2008). Therefore, injection of $FeSO_4$ into the trunk of date palm can be the best recommendation to achieve desirable results such as increment in plant Fe content. Iron injection into the trunk of the tree can supply adequate amounts of this essential element for plants regardless of high amounts of $CaCO_3$ and high pH of soil that can cause disorder in absorption and translocation of elements in plants (Fernandez-Escobar et al., 1993). The other researchers have reported an increase of Fe concentration in date leaves due to the Fe injection and application of FeEDDHA at rates up to 1000 g/tree had no effect on Fe concentration in leaves (Abo-Rady et al., 1987). Some other research also showed that the content of Fe in plant leaves increased due to Fe application through the localized placement method (Malakouti and Samar, 1998; Malakouti and Tabatabaei, 1999). Soil surface application of FeEDDHA caused the least increase in Fe concentration of leaves. Treatment 7 caused the most increase in Zn concentration of date leaves while other treatments showed less contents of Zn in leaves. The least amount of Zn concentration in leaves was also allotted to treatment 1 which is the control treatment, statistically. The increase of Zn concentration in treatment 7 is probably due to the high amounts of Fe concentration in these treatments. The antagonistic effect of Fe causes a decline in phosphorus concentration in leaves and then, Zn concentration is increased because of its reverse relation with phosphorus concentration (Bilsborough, 1993). Treatment 7 caused the highest increase in fruit set percentage, statistically. Other treatments showed a lower percentage of fruit set, the least percentage of fruit set was also allotted to treatment 1, statistically, which is the control treatment. The increase of Zn concentration in treatment 7 is probably due to the high amount of Fe concentration in these treatments. The antagonistic effect of Fe causes a decline in phosphorus concentration in leaves and then, Zn concentration is increased because of its reverse relation with phosphorus concentration The physiological roles of auxins have many implications in plant growth, such as cellular elongation and fruit development (Amarjit, 2004). In a number of species, including citrus, apples, pears and peaches, fruitlet and fruit set can be reduced or prevented (Amarjit, 2004). So, treatment 7 caused an increase in Fe and Zn concentration in leaves which increased fruit set.

Treatment 9 caused the highest increase in total sugar percentage of fruit, treatment 7, has statistically the same place as treatment 9, other treatments showed a lower percentage of sugar in fruit, the least amount of sugar in fruit was also allotted to treatments 2 and 3, statistically, which are FeEDDHA treatments. Iron has been shown to have an important role in the photosynthesis in plants (Archer, 1985; Nijjar, 1990; Zarrinkafsh, 1992) and sugar is the main product of photosynthesis. Injection of a solution

containing $FeSO_4$ with acidic pH into the trunk of tree, not only supplies enough amounts of available Fe for photosynthesis, but also improves absorption and translocation of other nutrient elements such as zinc, copper, manganese and phosphorus by reducing the pH of sap (Taiz and Zeiger, 1998).

Weight and lenght of fruit and stone, fruit weight, number and length of spathe, Brix, TSS, pH and reducing sugar showed no statistical differences in different treatments. Similar results were reported by Ahmed et al. (1987).

CONCLUSIONS

A precise investigation on obtained results showed that in most cases, injection of 25 g $FeSO_4$ $tree^{-1}$ caused the best results although the other methods and levels of iron fertilization showed similar results in some cases. Totally, the most desirable effects were seen in the injection treatments, but the treatment with 25 g $FeSO_4$ $tree^{-1}$ can be the best recommendation because it showed the best results. It is necessary to say that injection of a solution with a zero concentration of $FeSO_4$ did not cause any good results and was often similar to the control treatment, statistically. In the injection method, destination parts of the plant such as leaves, receive fertilizers solution regardless of soil $CaCo_3$ content. Soil surface application of Fe-EDDHA appeared as an improper fertilization method and allotted the lowest grade to itself, as well as the control treatment. In the recent method, fertilizers mainly remain unusable on the soil surface, whereas, nutrients should be present near the root to be absorbed more efficiently.

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Tables

Table 1. Effects of treatments on yield and yield component.

Treatment No	1	2	3	4	5	6	7	8	9
Yield (kg tree-1)	36.63 b	34.15 b	36.97 b	33.49 b	34.61 b	35.6 b	41.8 a	37.20 b	34.04 b
Brunch eight (kg)	3.12 bc	3.38 abc	3.01 c	3.56 ab	3.78 a	3.13bc	3.71 a	3.25 bc	3.18 bc
Fruit weight (g)	4.46 a	4.68 a	4.91a	4.80 a	4.93a	4.59 a	5.22a	4.68 a	4.58 a
% fruit set	47.42 c	52.57 ab	48.59 b	48.58 b	54.25 a	51.49 b	54.34 a	52.44 ab	52.66 ab
Fruit diameter (mm)	17.10 c	17.38 bc	17.77 abc	17.83 bc	18.16 ab	17.05 c	18.21 a	17.76 abc	17.49 abc
Stone diameter (mm)	5.978 ab	6.032 ab	6.133 a	5.863 b	6.059 ab	5.993 ab	6.054a	6.141 ab	5.908 ab
%sugar	74.2 ab	65.24 b	67.71 b	74.46 ab	71.77 ab	65.51 b	73.97ab	69.75 ab	81.62 a
Number of spathes	13.90 a	13.70 a	14.50 a	14.00 a	13.80 a	14.10 a	14.30 a	13.80 a	13.90 a
Spathe length (cm)	39.15 a	37.85 a	36.90 a	39.35 a	38.55 a	37.95 a	37.35 a	38.85 a	37.95 a
Spathe width (cm)	8.85 ab	9.02 ab	8.48 b	8.81 ab	8.48 b	8.82 b	9.12 a	8.80 ab	9.00 ab
Trunk perimeter (m)	1.63 c	1.73 ab	1.70 bc	1.70 bc	1.73 ab	1.73 ab	1.78a	1.63 c	1.80 a
Leave length (m)	3.228 b	3.338ab	3.289 ab	3.335 ab	3.335 ab	3.229 b	3.389 a	3.371 a	3.369 a
Leaflet length (cm)	41.71 bc	42.90 ab	42.93 ab	42.26 b	42.58 b	40.62 c	43.90 a	43.91 a	41.72 bc
Thorn length (cm)	7.08 b	7.68 a	7.41 ab	7.52 ab	7.43 ab	7.16 b	7.65 a	7.48 ab	7.49 ab
%N (leave)	1.27 a	1.10 b	1.19 ab	1.19 ab	1.22 ab	1.139 ab	1.19 ab	1.23 ab	1.16 ab

Economics of Date Palm Agriculture in the Sultanate of Oman, Current Situation and Future Prospects

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Abstract

Date palm is considered as the first crop in Oman in terms of number and distribution. Extra attention has been given to the tree by farmers and governmental agencies. The religious and social deep-rooted heritage enhanced its economic, nutritional and environmental benefits.

In the year 2007 the number of date palms reached about 8 million, producing about 261,000 tons, 13% more than the 2004 production. About 5.1 million trees (64%) produce table dates and 2.9 million produce dates for manufacturing. Date palm covers about 50% of the total agricultural land; representing 83% of fruit trees land in the Sultanate. Oman has more than 250 species of dates, some of good quality, commercial value, maturing date, nature of consumption and usage, diseases' resistance and yield. Some species are better than many well-known internationally; each area has its own best species.

Local human consumption of dates was estimated, during 2007, at about 132,000 tons, animal feed was about 53,000 tons. Average annual exports were 9,000 tons. There is a surplus of about 67,000 tons that can be processed for consumption or export, 30,000 tons of which are table dates.

Oman has given important attention and care to the tree related to applied scientific research, extension, development and investment. That included specialized research programs, improving production, tissue culture propagation, best agricultural practices, integrated pests management, gene banks, processing, marketing, storage, capacity building, and promoting date palm and its products. The Ministry of Agriculture (MoA) put a national strategy to improve date palm aimed to maximize economic, water use, social, and environmental returns on both the household and national levels. It contained an investment attitude based on the optimum benefit from all economical products of the blessed tree, using the up to date techniques available towards sustainable development. Recently His Majesty the Sultan issued an order to plant one million trees. The efforts are continuing to implement the order on comprehensive vision based on what is on the ground and what the future should be.

This article presents the role of the Agriculture and Fisheries Development Fund (AFDF) in supporting the efforts of maintaining and developing the tree during the last 5 years and those coming, and economic remarks on the optimum benefits from growing date palm in the Sultanate of Oman and future prospects.

INTRODUCTION

Date palm is considered as the first crop in Oman in terms of number and distribution. Extra attention has been given to the tree from the farmers and governmental agencies. The religious and social deep-rooted heritage enhanced its economic, nutritional and environmental benefits. The Arab region is the first in terms of number of trees and production of dates worldwide, the Sultanate of Oman is one of the most caring countries having environmentally comparative advantages to produce distinguished varieties of dates.

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DISCUSSION

Distribution of Date Palm in Oman

Table 1 shows estimations for the number of date palms according to Areas or Governorates, percentage to total during the period 2004-2008. Oman has about 6.5 million trees (productive female) producing an average of about 254,000 tons of dates. The production of 2008 grew by 36,000 tons (13%) more than 2004. AlBatinah is the biggest area in number and production; about 2.8 million trees (43% of the total) produce about 111 thousand tons, 44% of the total. AlSharqiya area came second in the number of trees, about 1.4 million (21% of total) and third in production, 43,000 tons (17%), while AlDakhliyah came second in production, 50,000 tons (20%) and third in number of trees, about 952,000 (14% of the total). These indicators need to be considered for any development programs or new future expansions.

The Most Productive Willayats

Table 2 shows the top ten Willayates producing dates, out of 61 Willayats for the period 2006-2008. During the years 2007 and 2008 Simail (AlDakhliyah) was the first, producing an average of about 21,700 tons (13.7%), Barkaa (AlBatinah) came second, it was the second during year 2006 and produced 21,600 tons (13.70%) despite the fact that they were so close. Ibri (AlDhahira) came third, producing an average of 18,800 tons of dates (12%). Tow Willayats, Nezwa and Simail, where in AlDakhliyah while six where in AlDakhliyah area.

The average total production of the top ten Willlayats for those three years, about 158,000 tons, represents about 60% of Oman's average total production (262,144 tons). This reflects the comparative advantages of each area and specific Willayat in date palm production and consequently reflects the appropriate environmental conditions and the best agricultural practices applied by farmers to produce efficiently.

The Highest Production Omani Varieties

Oman produces some of the best varieties of dates in the region. Some studies mentioned that there are more than 250 varieties (Ministry of Agriculture, 2003) while others mentioned that Oman has about 180 varieties (FadelAllah, 2003). Some of these varieties are excellent in their human nutrition and marketing values, others are good for animal feed or processing. The quality of dates depends on nutritional value, shape, time of ripeness, yield and productivity, age of first production, pests' resistance and environmental tolerance, purpose of consumption, and other criteria.

Generally date palms have been grown in most areas. Some references divide Oman into two main environmental areas, the coast which contains AlBatinah, Sur, Qurayyat and Msandam, the second is AlDakhliyah which contains ArRustaq, Nakhal, Samayiel, Finja, Izki, and other interior areas and AlDhahira, al Buraymi and AlSharqiyah. These two areas are relatively of different environments and each has its suitable varieties. Table 3 shows the top ten productive varieties during three years, 2006 to 2008. The 'UmSala' variety came first, producing an average of more than 35,000 tons (19% of total) during that period. 'Mibsily' came second, about 30,000 tons (16%), 'Khsab' came third, 27,500 tons (15%), 'Nakhal' (14%), 'Fardh', about 20,000 tons (11%), 'Shahal', 'Khlass' and 'Khnaizi' came sixth, seventh and eighth respectively.

The more attention and care producers give to the high quality varieties, nutritional value or shape and sizes preferred by consumers, in addition to best packing and postharvest practices, the more profit they get. Each factor contributes to achieving best marketing value and then the highest sale prices. A clear strategy needs to be built on continual evaluation of local varieties and using the best imported varieties in any expansion in the area or new farms to be established. Local and international demands, especially high value international markets, mainly Europe, should be considered in such expansions. A promotion plan for the local high quality varieties, as part of an efficient comprehensive marketing plan, is important. These fine products and their criteria need to

be introduced to all consumers on scientific bases.

Usage of Date Palms

Date palm growers, ranchers and farmers generally are working hard to make use of all market valued products of the tree. Local human consumption of date was estimated, during year 2008, at about 134,000 tons, animal feed was about 55,000 tons. Average annual exports were 7,000 tons. There was a surplus of about 71,000 tons; about 40.6 thousand tons can be processed and about 30.4 thousand tons are suitable for human consumption as table dates. These quantities need to be subject of developmental projects.

Productivity of Date Palm

After all, economic efficiency on a sustainable base is the ultimate goal of any production system. It should be the main objective of any decision maker to maintain the growing that crop. Many studies refer to the quality of date's varieties as one of the main elements, if not the most important one, that affects its marketing profitable prices, in addition to the quantity of annual production of dates, to achieve economic profit.

Table 4 shows the average annual yield of date palms across the Sultanate areas and governorates during the period 2004 to 2008. AlDakhliyah area was leading where its trees average a yield reached about 51 kg per tree annually. AlBatinah and AlDhahira each got about 41 kg per tree on average, Muscat and Braimi about 33 kg each, and finally AlSharqiya and Msandam trees produced about 30 kg in average each.

Although the Omani average yield was about 39 kg per tree, yield on the Willayats level during the 2008 season showed another side of the picture. Nakhal Willayat (AlBatinah) gave 104.2 kg per tree. Samail (AlDakhliyah) date palms produced an average of 85.12 kg per tree. This is a good and promising productivity and one may build on them. Wadi ElMaawil (AlBatinah) came third, producing an average of 64 kg per tree, and then came Nezwa (AlDakhliyah) with about 61 kg per tree.

Because of the high current cost of production most yields, within 55-25 kg per tree, are considered to be medium or low. Others, that go up to 65 kg, are acceptable although there should be an institutional work to improve current productivity. Especially, there are possible chances such as those of Nakhal Willayah (104 kg per tree) to be consistent with the comparative advantages of the Sultanate and the high level of care that has been given to the date palms.

There are many factors that affect the yield of date palm, mainly variety, age, irrigation system, environmental conditions, type, quality and timing of agricultural operations and some other minor factors. Special attention needs to be given to the irrigation systems and their economics because of the common water limitations, high costs of water, and the technical and economical efficiency of modern irrigation, which are improved by many scientific studies, related to date palm production. Although there are some previous studies related to Omani date palm, there is a serious need to continually update those studies and doing new comprehensive technical-economical feasibility studies for modern irrigation systems and their use on different important crops including date palm.

Role of AFDF

Oman has given important attention and care to the tree related to applied scientific research, extension, development and investment. That included specialized research programs, improving production, tissue culture propagation, best agricultural practices, integrated pests management, gene banks, processing, marketing, storage, capacity building, and promoting date palm. The Ministry of Agriculture has a national strategy to improve date palm aimed to maximize economic, water use, social, and environmental returns on both the household and national levels. It contained an investment attitude based on the optimum benefit from all economical products of the blessed tree, using the up-to-date techniques available towards sustainable development. Recently His Majesty the Sultan issued a decree to plant one million trees. The efforts are

continuing to implement the decree in a comprehensive vision based on what is on the ground and how the future should be.

The Agriculture and Fisheries Development Fund (AFDF) is supporting the efforts of maintaining and developing the tree during the last 5 years and those coming. The AFDF financed 19 developmental projects. The projects have cost more than 4 million Omani Riyal (about US$ 10.5 million). They dealt with research, extension, IPM, introducing new techniques, modern irrigation systems, and other development aspects. Many technical and socio-economic achievements are recorded. New promising projects are coming.

Million New Date Palms

Adding a million new date palms will affect the future of the tree and its contribution to the agricultural system and economy in addition to the environmental system of Oman. Farmers, workers and investors as producers will make benefits also consumers, consequently, will gain via products biodiversity, better quality, and price stability and reduction. Also the Gross National Income will be positively affected and the trade balance through exports and new markets. The date industry in general will benefit with the implementation of the decision built on a comprehensive technical and economic feasibility study.

The technical parts related to the selection of appropriate locations which have qualified infrastructure, a complete soil analysis, using its results to build the nutrition program, insuring availability and quality of irrigation water for adequate long time use as a base to select the optimum modern irrigation system. The number of qualified workers, their level of experience, and sustainability to insure continued best agricultural practices during its productive age making use of growing experiences. It is crucial to depend on, even to some extents, local labor and employ Omani capacities especially youth and women as much as possible. The technical and practical capabilities of local workers will insure his/her sustainable ability to apply the best adopted new modern techniques in the new farms.

Using machinery, as much as possible and needed, in the production process or postharvest, needs to be built on a national strategy taking into account actual needs, available resources and previous experiences, and a level of knowledge or ability of related beneficiaries. An appropriate technology means technically possible, economically feasible, and socially acceptable to be adapted to suit local environment conditions.

Best varieties have to be carefully selected to meet local and international markets requirements. It is better to focus on limited best local varieties which pass comprehensive evaluation and new imported promising varieties not to spread shallow losing concentration on economically valuable ones.

Financial and Economic Considerations

The social and religious care of the blessed tree in Arab society may cause underestimation of some, or most, financial and economic components of the growing date palm project despite their clear importance to the producers. It is critical to draw an implementation plan standing on reliable financial information. An accurate primary source of financial information, mainly the cost system, is very important and may change the whole picture. Producers have to be aware of some of the basic financial issues such as, statement of cash flow (developed from the income statement and balance sheet), required working capital, assets, income and net income. Also he/she needs to learn a bit about cost, depreciation, market and book value, profitability ratios, clear edge and details for all economic components of the project, its timing, risks and uncertainty, and expected returns.

Date palm economics in Oman have a significant shortage in availability and/or updating its data-base, mainly all kind of costs, expected returns and economic profit from that agricultural activity. Costs are usually related to the size of farms, varieties,

agricultural system and production techniques including level of machinery, irrigation system, and postharvest services. There are additional critical costs, usually affecting the sustainability of such an activity of planting a million new palms, such as the cost of processing dates, their products, and other products of the palm. Date palm is a multi-benefits crop; all of it can be of market value contributing to the economic profit function.

Suggested Cost Structure

Limited published reports and studies focused on costs and returns of date palm production in Oman, either area or tree base. In the year 2000 a study showed that the average cost of one feddan was about 354 Omani Riyal (US$ 920). The highest average cost was for AlSharqiyah area, 381 OR (US$ 991), the lowest at Msandam, 233 OR (606 US$). AlBatinah area got the same average as the Sultanate. There is a project to update that study.

One of the common methods of costs reporting is to calculate the actual cost of a selected sample of farms that have approved book keeping as part of their accounting system within a certain period of time. Direct reporting of expenditures and returns at their time, for appropriate time series, is another method. A third way is to use documented cost-benefit data from an investment company or specialized research institute and readjust their elements according to the local conditions.

Costs structure shall contain fixed costs such as cost of land, its rent, and constructions such as offices, housing, services, fences, wells and water reserves, irrigation system pipelines, seedlings, all can be calculated as annual depreciation. Costs of agricultural machinery, tools, pumps, sprayers, generators, and other equipments all can be calculated on annual depreciation using the common methods of calculations depending on their productive age and cost. Cost of permanent staff is part of fixed costs as well.

Operation or variable costs, that are required to provide production inputs, such as fertilizers, pesticides and herbicides, fuel, electricity, pollination if not available on farm, sacks, maintenance and spear parts, temporary workers wages, and any other variable costs. All technical specifications of the project components need to be taken care of in order to reach an acceptable cost estimation close to the reality.

Marketing

Arab countries produce about 73% of the global date production in the year 2008, which was about 6.4 million ton. Only 2.3% of the production of the year 2002 was exported. Although Asian countries imported 70% of world trade, its value was about 36% of the total market value, in average of US$ 345 per ton, while European countries imported 13% of marketed dates with a value that reached 46% of the total sales, in average US$ 1760 per ton (Oihabi, 2009).

Although Omani dates exports were so small for the last three years, 2.33, 4.10, and 9.33 thousand tons valued 175.66, 181.70, and US$ 269.85 million for the years 2005, 2006, and 2007 respectively, in annual average 3.29 thousand tons at US$ 4.62 million, they can still be improved in quantity and quality to enter the international market, mainly Europe, if the suitable varieties are produced and marketed properly.

Pilot Project

It is recommended to implement a commercial agriculture production project built on a technical and economical feasibility study via three stages. It starts with a large scale applied research model, especially in case of unavailability of updated comprehensive research recommendations issued by related research centers of the country for that specific location and that main crop and other crops required for suitable crop rotation. Then a pilot project that makes use of research results can simulate commercial project techniques and then there is a third stage of commercial project building based on results of the two previous stages and developing the components of the feasibility study of the project. This concept helps in successful performance for many practical reasons which

are not the subject of this article.

To implement a project of a million new date palms in Oman, the feasibility study may benefit from good research and investment experiences either in Oman or neighboring countries. Decision makers may start with the second stage, the pilot project. Establishing a "Model Farm" in selected areas using a full technical package, including top varieties, either suitable for human consumption or processing, modern irrigation system, latest appropriate technologies, adopting best agricultural practices with efficient qualified management that coordinates all efforts of researchers, extension experts, farmers, and investors within an integrated program.

The expansions in areas and numbers of farms should be according to a suitable time frame depending on results and achievements. The work can be done in two ways one for new farms while the second towards replacement of some current nonproductive, inefficient, and low quality and quantity farms.

The study needs to pay special attention to the marketing, processing of dates, their products and other products of the tree, and all postharvest alternatives and requirements. Also the study may refer to organic production of dates.

The private sector, either people involved in the business now or newcomers, has to be encouraged to participate in such a project in one way or another. The buildup of experience is crucial in agro-business and in date palm production the importance is of no doubt.

SUMMARY AND RECOMMENDATIONS

1) Oman has about 6.5 million date palms, productive females, across the Sultanate producing about 267,000 ton of dates annually some of high quality varieties.
2) Oman is giving important attention to the tree built on a comprehensive national strategy related to applied scientific research, extension, and investment towards sustainable development.
3) The AFDF financed 19 developmental projects costing about US$ 10.5 million. Many technical and socio-economic valuable achievements are recorded. New promising projects are coming.
4) Although the Omani average yield was about 39 kg per tree, some Willayat, such as Nakhal gave 104.2 kg per tree and Samail gave 85.12 kg per tree in the year 2008. There should be an institutional work to improve current productivity.
5) Although Omani dates exports were so small for the last three years, 3.29 thousand tons valued US$ 4.62 million, they can still be improved in quantity and quality to enter the international market, mainly Europe.
6) Date palm economics have a significant shortage in availability and/or updating the data-base, mainly all kinds of costs, expected returns and economic profit from that agricultural activity.
7) Adding a million new date palms will affect the future of the tree and its contribution to the agricultural system, economy, and the environmental system of Oman.
8) The million new date palms project may be built on a technical and economical feasibility study via three stages. Starting with large scale applied research model, then a pilot project that makes use of research results and simulates commercial project techniques, then a third stage of commercial project building on results of the two previous stages and developing the components of the study.
9) The expansions in areas and numbers of farms should be according to a suitable time frame depending on results and achievements.
10) There are many factors that affect the yield of date palm, mainly variety, age, irrigation system, environmental conditions, type, quality and timing of agricultural operations and some other minor factors.
11) Special attention needs to be given to the irrigation systems and their economics because of the common water limitations, high costs of water, and the technical and economical efficiency of modern irrigation, which are improved by many scientific studies, related to date palm production.

12) Although there are some previous studies related to Omani date palm, there is a serious need to continuously updating those studies and doing new comprehensive technical-economical feasibility studies for modern irrigation systems and their use on different important crops including date palm.
13) The study needs to pay special attention to the marketing, processing of dates, their products and other products of the tree, and all postharvest alternatives and requirements. Also the study may refer to organic production of dates.
14) The private sector, either people involved in the business now or newcomers, has to be encouraged to participate in such a project in one way or another. The buildup experience is crucial in agro-business and in date-palm production the importance is of no doubt.
15) Continued support to applied scientific research is required to improve its mechanisms and sustain its results. The sector needs more coordination between researchers, related beneficiaries, extension agencies, and investors to apply and evaluate the suitable achieved results.
16) It is important to strengthen date palm producers and traders via institutionalizing their work. A national electronic network may start the coordination to reach the appropriate organization shape making use from related pioneer experiences in the Sultanate and the region.

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Tables

Table 1. Number of date palm distribution (2008), production of date (period 2004-8) in Oman*.

Area	Production during years (ton)							Date palms 2008	
	2004	2005	2006	2007	2008	Av. 5 years	Prod.	No.	%
AlBatinah	105929	112095	113957	111216	111407	110920	44	2817190	43.43
AlDakhliyah	44006	47580	51414	52554	54497	50010	20	1397010	21.35
AlSharqiya	37295	40426	42592	46284	48920	43103	17	952428	14.68
AlDhahira	29516	36327	35604	35401	27136	32797	13	550545	8.48
Muscat	10283	10935	10780	10795	11574	10873	4.3	338674	5.22
Msandam	3874	4044	4258	4428	4521	4225	1.6	265012	4.08
Braimi	--	--	--	-	8704	-	-	144381	2.22
Dhufar	132	132	132	132	132	132	0.05	21388	0.03
Total	231035	251538	258738	260810	266893	253803		6486628	

* Reference: Ministry of Agriculture-Sultanate of Oman Production of Dates Report 2007and 2008.

Table 2. Top ten most productive willayats for the period 2006-2008 in Oman (ton)*.

sq	Willayat	Area	Production (ton)			Average 3 years	% of total
			2006	2007	2008		
1	Simail	AlDakhliyah	20779	21660	22686	21708	13.75
2	Barkaa	AlBatinah	23755	21071	20023	21616	13.70
3	Ibri	AlDhahira	19226	18535	18632	18797	11.91
4	Mudhabi	AlSharqiyah	16506	16863	20408	17926	11.36
5	AsSuwayq	AlBatinah	16572	16800	16844	16739	10.60
6	Sohar	AlBatinah	13241	13875	14362	13826	8.76
7	Nezwa	AlDakhliyah	12975	12553	12465	12664	8.02
8	Nakhal	AlBatinah	12408	11507	12078	11998	7.60
9	Musnaa	AlBatinah	11102	11221	10859	11061	7.01
10	Khaborah	AlBatinah	9087	9506	9827	9473	6.00
Total			157657	155598	160192	157816	

* Reference: Ministry of Agriculture-Sultanate of Oman Production of Dates Report 2007 and 2008.

Table 3. Top ten most productive varieties during the years 2006-2008 in Oman (ton)*.

Sq.	Variety	Production of dates during years (ton)			Average 3 years	% of total
		2006	2007	2008		
1	UmSal	34991	35465	35218	35225	19
2	Mibsily	30095	29698	31175	30323	16
3	Khsab	27472	27181	27944	27532	15
4	Nakhal	27603	25069	24639	25770	14
5	Fardh	19475	18956	20482	19638	11
6	Shahal	11900	12258	12602	12253	7
7	Khlass	12353	12134	12658	12382	6.7
8	Khnazi	11263	11135	11264	11221	6
9	Madloki	5594	4896	5152	5214	2.82
10	Misly ** irny	5068	4852	5056	4954	2.7
Total		185814	181644	186190	184549	

* Reference: Ministry of Agriculture-Sultanate of Oman Production of Dates Report 2007 and 2008.

Table 4. Average date palm yield (kg of date) across the willayats (Period 2004-2008)*.

sq	Area	Annual Productivity (kg per tree)					Average
		2004	2005	2006	2007	2008	5 years
1	AlDakhliyah	46	45	54	55	57	51.4
2	AlBatinah	38	50	40	39	39	41.2
3	AlDhahira	36	32	44	43	49	40.8
4	Muscat	30	39	32	32	34	33.4
5	Braimi	-	-	-	-	33	33
6	AlSharqiyah	27	28	30	33	35	30.6
7	Msandam	27	29	29	31	31	29.4
8	Dhufar	6	6	6	6	6	6
Average/Oman		35	39	40	39	41	38.8

* Reference: Ministry of Agriculture-Sultanate of Oman Production of Dates Report 2007 and 2008.

Extending Harvest Season, Improving Fruit Quality and Shelf Life of 'Barhee' Date Palm by Preharvest Sprays

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Keywords: date palm, preharvest sprays, ripening, storagability

Abstract

The present study was conducted in order to extend the harvest season and to maintain fruit quality for better marketability of 'Barhee' date palms growing in Riyadh in Saudi Arabia. Palms were treated by preharvest foliar sprays at the hababouk stage and at the beginning of fruit color break with 10 ppm from a new cytokinin related substance (CPPU or N-(2-chloro-4-pyridinyl)-N'-phenyl urea), known as cytofex, 8 mM putrescine (Put) and 50 ppm each of GA_3, NAA, benzyl adenine "BA" and salicylic acid "SA". The harvest date was delayed by one month with NAA and GA_3, three weeks with Put and SA, and by two weeks with CPPU and BA from the commercial harvest date. All treatments decreased skin color intensity and carotenoids content and increased acidity as compared with the control. The fruits turning to the postharvest rutab stage during storage at 0°C and 85-90% RH were reduced by all treatments. The GA_3, NAA and Put treatments had a significantly higher effect in extending the shelf life and decreasing the fruit weight loss percent than the other treatments. It is concluded that the sprayed growth regulators had a positive influence on extending the harvest season and the shelf life of 'Barhee' dates without any deterioration in fruit characteristics before and during cold storage.

INTRODUCTION

Date palm fruits (*Phoenix dactylifera* L.) are highly demanded and consumed throughout the world, especially in the Middle East. According to FAO (2009), Saudi Arabia is considered the third country of the top ten date producers (982,546 tones). A small quantity of certain date cultivars (such as 'Barhee') are harvested and consumed at the khalal stage when they reach full maturity (partially-ripe) and are yellow, pink, or red in color (according to the cultivar). In this stage these cultivars are less astringent than other cultivars that are only harvested when they are fully ripened and are yellow, pink, or red in color. However, once ripened, these cultivars have a short shelf life (Hong et al., 2006). In the mean time, dates consumers are looking for fruits with greater color and bigger size. The small fruit size of 'Barhee' dates is another limiting factor that influences its marketing. Thus; it would be beneficial to improve quality characters and to prolong the khalal stage of these cultivars in order to expand their marketing ability. Plant growth regulators play an important and major role in regulating fruit growth and development. Some of these substances are used in controlling ripening of dates as well as improving the fruit quality, which act for increasing the income and the revenues of farmers. NAA was found to increase fruit size, weight and delayed ripening of dates (Aljuburi et al., 2000; Aljuburi et al., 2001; Aboutalebi and Beharoznam, 2006). Also, GA_3 increased fruit weight, and delayed fruit ripening (Moustafa and Seif, 1996). Benzyl adenine and cytofex increased the fruit size and delayed chlorophyll breakdown and fruit aging (Stern et al., 2006). Polyamines, mainly diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm), are polycationic compounds of low molecular weight that are present in all living organisms. They have been proposed as a new category of plant

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growth regulators that are purported to be involved in a large spectrum of physiological processes, such as embryogenesis, cell division, morphogenesis, and development (Liu et al., 2007). In addition, putrescine was found to retard color change and decrease firmness loss, delay ethylene production and respiration rate (Martinez-Romero et al., 1999; Valero et al., 1999), which resulted in reducing senescence rate after harvest (Martınez-Romero et al., 2002). In addition, salicylic acid was reported to retard ethylene synthesis (Leslie and Romani, 1988). Also, it has been recognized that salicylic acid is required in the signal transduction for inducing systemic acquired resistance against some pathogenic infections (Vernooij et al., 1994). Sayyari et al. (2009) indicated that salicylic acid improved fruit quality during cold storage of pomegranate.

In accordance to the previously mentioned, the present study was conducted in order to investigate the effect of spraying NAA, GA_3, cytofex (CPPU), putrescine (Put), benzyl adenine (BA) and salicylic acid (SA) on improving fruit quality of 'Barhee' dates before and during cold storage.

MATERIALS AND METHODS

Plant Materials and Treatments

The present study was conducted during the 2008 and 2009 seasons at the Research and Agricultural Experimental Station at Dirab, King Saud University, Saudi Arabia on 'Barhee' date palms (*Phoenix dactylifera* L.). The palms were planted 10×10 m apart and subjected to the same cultural practices usually done in the orchard. Organic manure, calcium super phosphate and potassium sulfate were applied in December of each season at the rate of 15, 1 and 1.5 kg per palm, respectively. Also, ammonium nitrate at the rate of 3 kg/palm was applied at three equal doses; mid-February, mid-April and mid-May of each season. Eleven palms were selected as uniform as possible and bunches were pollinated from the same male palm tree. Bunches were sprayed at both hababouk and the beginning of fruit color break stages with gibberellic acid (GA_3), naphthalene acetic acid (NAA), putrescine (Put), salicylic acid (SA), N-(2-chloro-4-pyridinyl)-N'-phenyl urea (CPPU, 'cytofex') and benzyl adenine (BA). Palms were subjected to seven foliage treatments with three replicates per treatment and three bunches for each replicate (i.e., 7 treatments × 3 replicates × 3 bunches = 63 bunches on 11 palm trees). Treatments arranged in a complete randomized design were as follows: 1) water only (control), 2) NAA (50 ppm), 3) GA_3 (50 ppm), 4) cytofex "CPPU" (10 ppm), 5) PUT (8 mM), 6) BA (50 ppm) and 7) SA (50 ppm).

The surfactant Nourfilm (produced by Alam Chemica Co.) at the rate of 40 cm/100 L water was added to all sprayed substances in order to obtain the best penetrating results and bunches were sprayed once in the early morning. In order to determine the effect of the different treatments on fruit physical and chemical characteristics, a sample of ten strands were randomly collected from each bunch/replicate during both seasons at the commercial harvest date when the control fruits reached full maturity and yellow color.

Physical Properties

Fruit physical properties were determined at harvest; fruit and pulp weight (g), fruit diameter and length (cm), fruit volume (cm^3). Also, ground fruit color (assessment of color change) was estimated by giving five degrees of color stage as follows; (1) = 100% green, (2) = 25% yellow, (3) = 50% yellow, (4) = 75% yellow and (5) =100% yellow. Fruit skin color in the 'Barhee' cultivar was assessed visually and recorded on a scale from 0 (no color change) to 5 (complete change).

Chemical Properties

Fruit chemical properties were determined at harvest; the percentage of total soluble solids was measured by a hand refractometer, acidity (%) was determined by titration according to AOAC (1995), Carotenoids and total chlorophyll contents

(mg/100 g peel fresh weight) were achieved by the method of Moran and Porath (1980), as 80% acetone extract was colorimetrically assayed at 650 nm, for total chlorophyll and 440 nm for carotene using a spectrophotometer and the percent of reducing, non-reducing and total sugars were determined according to the method of Malik and Singh (1980).

Storagability

To study the effect of the different treatments on fruit storage ability and shelf life, a second fruit sample of 25 strands was randomly collected from each replicate when every treatment reached full maturity and yellow color and harvest date for each treatment in both seasons was recorded. Strands were kept at 0°C and 85-90% relative humidity for 45 days and the incidence percentages of fruit rutab, decay and weight loss were determined every 15 days during the cold storage.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) to detect the treatment effect. Mean separation was performed by using the least significant difference (LSD) at the $p \leq 0.05$ level. The data were analyzed using a statistical analysis system (SAS, 1990).

RESULTS AND DISCUSSION

Fruit Physical Characteristics

Data obtained in both seasons are presented in Table 1. All sprayed substances (except SA) significantly increased fruit weight, diameter, length and volume and pulp weight when compared with the control. GA_3 and Put sprays resulted in a significantly higher effect in increasing fruit weight, length and volume and pulp weight than NAA in the first season with no significant difference between them. However, in the second season no significant differences were obtained among the NAA, GA_3, CPPU, Put and BA treatments. In the mean time, fruit diameter did not significantly differ among the previously mentioned compounds in both seasons. In addition, data of both seasons showed a marked delay in the fruit green color break by all sprayed compounds as compared with the control. Fruit green color break was significantly lower by NAA than BA and CPPU sprays in the first season. Moreover, no significant difference between BA and CPPU on the one hand and between NAA, GA_3, Put and SA treatments on the other hand was found. In the second season, the NAA and SA had a significantly higher effect on retarding fruit green color break than BA, Put and CPPU with no significant differences between BA, putrescine and cytophex on the one hand and between NAA, GA_3 and SA on the other hand was obtained.

In general, the data obtained in our study showed that all sprayed growth regulators had positive influences in increasing fruit weight, diameter, length and volume and pulp weight and retarded fruit green color break of 'Barhee' dates. This increment in fruit physical characteristics was also reported by numerous investigations working on different fruit trees (Aljuburi et al., 2000, 2001; Stern et al., 2006; Aboutalebi and Beharoznam, 2006). The improvement in fruit physical properties as a result of the different growth regulators treatments might be due to their influence in enlarging the cells' size and enhancing the strength of carbohydrate sink, thus increasing fruit size and weight. Similarly, Valero et al. (2002) and Liu et al. (2007) reported that polyamines are essential for cell growth and differentiation and their intracellular concentrations increase during periods of rapid cell proliferation. Also, the role of putrescine in delaying fruit color break was reported by Serrano et al. (2003) and Martinez-Romero et al. (2002). Benzyl adenine and cytofex sprays were found to delay chlorophyll breakdown and fruit aging (Stern et al., 2006).

Fruit Chemical Characteristics

The effect of the various treatments on fruit chemical parameters at harvest are

presented in Table 2. The data obtained showed that fruit acidity was significantly increased by spraying NAA and SA (in both seasons), Put (in the first season) and GA_3 (in the second season) when compared with the unsprayed control. No significant difference was obtained among the previously mentioned treatments during both seasons. In addition, fruit chlorophyll increased significantly with all treatments during both seasons. Spraying SA had a significantly higher effect in increasing the fruit chlorophyll content than BA and cytofex in the first season only, whereas, no significant difference was obtained among SA, NAA, GA_3 and Put. In the second SA and NAA had a similar and higher effect in increasing peel chlorophyll content than BA, cytophex and putrescine with no significant differences obtained among the BA, cytophex and putrescine treatments. A significant increase in the fruit non-reducing sugars was obtained by NAA, GA_3, SA and Put sprays (in both seasons) and CPPU (in the first season), with no significant differences found among the previously mentioned treatments during both seasons. Total soluble solids content decreased by NAA, SA and putrescine sprays (in both seasons) and GA_3 (in the second season) as compared with the control, with no significant difference obtained among the previously mentioned treatments during both seasons. Moreover, the fruit reducing sugars also decreased with all treatments (except BA) during both seasons with no significant differences obtained among them in the first season. However, in the second season the highest decrease of reducing sugars content was obtained by SA sprays. In addition the NAA, Put, GA_3 and CPPU treatments showed no significant difference among them in the second season. Fruit total sugars were decreased by spraying NAA, SA, CPPU and Put in both seasons and by GA_3 in the first season. In the mean time, all sprayed substances decreased the fruit carotene content in both seasons as compared with the water sprayed control.

The high increase in fruit acidity, non-reducing sugars and total chlorophyll and the decrease in fruit TSS and carotene contents obtained in our study by NAA, GA_3 and CPPU application might translate their influence in retarding the fruit ripening process as mentioned before by (Moustafa and Seif, 1996; Aljuburi et al., 2000) working on date palm fruit. Similarly, the role of putrescine and salicylic acid in delaying fruit ripening was indicated. The main effect of putrescine is lowering the ethylene production and respiration rate as well as inducing mechanical resistance (Martinez-Romero et al., 2002). Salicylic acid was reported to activate the metabolic consumption of soluble sugars to form new cell constituents as a mechanism for stimulating plant growth, and might also be assumed to inhibit the polysaccharide-hydrolyzing enzyme system and/or accelerate the incorporation of soluble sugars into polysaccharides (Akhodary, 2004). The previously mentioned might be leading factors to the role of salicylic acid in retarding fruit ripening.

Harvest Date and Storagability

The effect of different foliar sprays on fruit harvest date (ripe) in both seasons is presented in Table 3. The ripening period was prolonged by 38, 30, 21, and 24 days for NAA and GA_3, SA and Put sprays, respectively, as compared with the water sprayed control. CPPU had a moderate effect on delaying fruit ripening, whereas BA delayed fruit ripening by only 10 days in comparison with the control.

As for the effect of the different spray treatments on fruit storagability determined as the percent of rutab incidence of fruits and weight loss in both seasons the results are presented in Table 3. The data obtained showed that rutab percent during 45 days of storage at 0°C and 85-90% RH was significantly reduced by all treatments. NAA resulted in the lowest rutab percent, followed by GA_3, Put, SA, CPPU and BA sprays. The storage life of 'Barhee' dates treated with NAA was extended by 30 day without incidence of any fruit rutab occurred, however, by 45 days the fruit reached a rutab percentage of 12.76 and 11.32 in the 2008 and 2009 seasons, respectively. Regarding the effect of the different treatments on fruit weight loss during storage, the data in Table 3 revealed that the percentage of fruit weight loss tended to decrease with all sprayed substances when compared with the control. Weight loss is an important factor that limits postharvest fruit

storage life (Adato and Gazit, 1974). Fruits going into ripening and senescence are mainly characterized by disintegration of organelle structures, intensive loss of chlorophyll and proteins, membrane leakage and breakdown of cell wall components leading to loss of tissue structure (Paliyath and Droillard, 1992; Buchanan-Wollaston, 1997). Ethylene is known to have a primitive effect on ripening and senescence processes (Abeles et al., 1992). From the results above, decreasing fruit deterioration (reduction of rutab incidence and weight loss) might be due the effect of the sprayed substances on regulating ethylene production or action, and thus, slowing down fruit senescence. Polyamines are well-known regulators of growth and differentiation and may compete directly with ethylene for their common precursor S-adenosylmethionine, thus reducing or even nullifying ethylene emission in the final days of fruit growth (Bagni and Torrigiani, 1992). Also, putrescine application was found to reduce the activities of fruit softening enzymes in the skin and pulp tissues (Khana et al., 2007). SA significantly maintained fruit firmness and lowered fruit decay during cold storage (Wanga et al., 2006).

CONCLUSIONS

In general, this experiment showed the effectiveness of all preharvest treatments on 'Barhee' date palm fruit quality, but SA, NAA, Put or GA_3 treatments were better than CPPU or BA treatments. The preharvest NAA, GA_3 and SA treatments prevented fruit rutab (softening) and decreased weight loss and TSS. These treatments can be easily used instead of laborious postharvest treatments to improve 'Barhee' date palm fruit quality.

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Tables

Table 1. Effect of growth regulators on physical characteristics of 'Barhee' fruits during the 2008 and 2009 seasons.

Treatment	Fruit parameters					
	Weight (g)	Volume (cm^3)	Length (cm)	Diameter (cm)	Pulp weight(g)	Ground color
			2008			
Control	9.21_{c}	8.80_{d}	2.48_{d}	2.04_{b}	8.54_{d}	5.00_{a}
NAA	12.68_{b}	12.17_{bc}	3.07_{bc}	2.60_{a}	11.38_{bc}	3.60_{c}
GA_3	14.86_{a}	14.63_{a}	3.53_{a}	2.65_{a}	13.75_{a}	4.00_{bc}
CPPU	13.90_{ab}	13.50_{ab}	3.42_{ab}	2.72_{a}	12.67_{ab}	4.22_{b}
Put	14.47_{a}	14.00_{a}	3.48_{a}	2.63_{a}	13.83_{a}	4.04_{bc}
BA	13.84_{ab}	13.33_{ab}	3.18_{ab}	2.47_{ab}	12.70_{ab}	4.40_{b}
SA	10.80_{c}	10.45_{cd}	2.80_{cd}	2.45_{ab}	9.81_{cd}	4.00_{bc}
			2009			
Control	9.97_{b}	9.87_{b}	2.93_{b}	2.30_{c}	8.98_{b}	5.00_{a}
NAA	13.60_{a}	13.17_{a}	3.37_{a}	2.64_{ab}	12.61_{a}	4.02_{d}
GA_3	13.86_{a}	13.83_{a}	3.33_{a}	2.69_{ab}	12.84_{a}	4.12_{cd}
CPPU	14.90_{a}	14.50_{a}	3.42_{a}	2.76_{a}	13.67_{a}	4.24_{bc}
Put	14.17_{a}	14.00_{a}	3.60_{a}	2.67_{ab}	12.83_{a}	4.24_{bc}
BA	15.34_{a}	14.83_{a}	3.38_{a}	2.71_{ab}	14.03_{a}	4.36_{b}
SA	10.85_{b}	10.85_{b}	2.98_{b}	2.49_{bc}	9.92_{b}	4.06_{d}

Values within a column with the same letter are not significantly different ($p<0.05$).

Table 2. Effect of growth regulators on chemical characteristics of 'Barhee' fruits during the 2008 and 2009 seasons.

Treatment	Fruit parameters						
	Acidity (%)	TSS (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Total chlorophyll (mg/100 g)	Carotene (mg/100 g)
				2008			
Control	0.31_{c}	33.4_{ab}	21.64_{a}	8.53_{d}	30.17_{a}	2.94_{d}	8.47_{a}
NAA	0.49_{ab}	30.3_{cd}	18.38_{b}	9.97_{ab}	28.35_{b}	5.74_{ab}	4.04_{b}
GA_3	0.43_{abc}	31.8_{bc}	18.47_{b}	9.68_{b}	28.15_{b}	4.86abc	3.75_{b}
CPPU	0.38_{bc}	32.2_{ab}	18.70_{b}	9.69_{b}	28.39_{b}	4.68_{bc}	3.19_{b}
Put	0.48_{ab}	29.4_{d}	18.54_{b}	9.48_{bc}	28.02_{b}	5.38_{abc}	3.74_{b}
BA	0.38_{bc}	33.6_{a}	19.89_{ab}	8.67_{cd}	28.56_{ab}	4.28_{c}	4.23_{b}
SA	0.53_{a}	30.4_{cd}	17.47_{b}	10.78_{a}	28.15_{b}	6.03_{a}	3.29_{b}
				2009			
Control	0.39_{b}	32.8_{a}	20.28_{a}	9.23_{c}	29.43_{a}	2.18_{c}	7.83_{a}
NAA	0.58_{a}	30.0_{b}	17.63_{b}	10.83_{ab}	27.46_{bc}	7.37_{a}	4.76_{bc}
GA_3	0.61_{a}	27.6_{b}	17.57_{b}	10.93_{a}	28.40_{ab}	6.15_{ab}	3.28_{c}
CPPU	0.48_{a}	31.9_{a}	17.45_{b}	9.74_{bc}	27.19_{bc}	4.83_{b}	4.14_{bc}
Put	0.42_{b}	28.4_{b}	17.10_{bc}	10.37_{ab}	27.47_{bc}	4.87_{b}	4.76_{bc}
BA	0.42_{b}	31.6_{a}	18.17_{ab}	9.93_{abc}	28.10_{ab}	4.43_{b}	5.48_{b}
SA	0.63_{a}	28.4_{b}	14.93_{c}	10.83_{ab}	25.76_{c}	7.86_{a}	4.63_{bc}

Values within a column with the same letter are not significantly different ($p<0.05$).

Table 3. Effect of growth regulators on harvest date, fruit rutab and weight loss percent during cold storage of 'Barhee' fruits during the 2008 and 2009 seasons.

Treatment	Harvest date	% Rutab after days			% weight loss		
		15	30	45	15	30	45
			2008				
Control	1/8	12.43	32.65	86.98	3.63_{a}	6.76_{a}	13.24_{a}
NAA	8/9	0.0	0.0	12.76	2.00_{c}	3.42_{c}	9.45_{bc}
GA_3	30/8	1.32	4.65	16.25	2.23_{c}	4.06_{bc}	8.21_{c}
CPPU	18/8	4.34	13.54	22.34	2.82_{b}	4.79_{bc}	12.22_{ab}
Put	21/8	1.23	5.87	12.43	2.35_{bc}	4.04_{bc}	10.80_{abc}
BA	10/8	6.65	14.43	37.33	2.76_{b}	5.32_{ab}	12.55_{ab}
SA	24/8	2.67	5.33	24.33	2.17_{c}	5.12_{abc}	9.30_{bc}
			2009				
Control	27/7	16.65	28.53	95.00	3.84_{a}	8.5_{a}	13.46_{a}
NAA	4/9	0.0	0.0	11.32	2.21_{b}	4.42_{b}	8.45_{b}
GA_3	2/9	2.33	7.67	18.46	2.33_{b}	5.07_{b}	9.21_{ab}
CPPU	14/8	5..67	12.76	31.38	2.76_{b}	5.39_{b}	10.22_{ab}
Pyt	18/8	1.67	6.72	19.45	1.95_{b}	4.74_{b}	8.80_{b}
BA	12/8	3.21	18.65	33.33	2.78_{b}	5.08_{b}	10.55_{ab}
SA	22/8	4.67	4.42	20.33	2.21_{b}	4.28_{b}	9.30_{ab}

Values within a column with the same letter are not significantly different ($p<0.05$).

Metaxenic Effects as Related to Hormonal Changes during Date Palm (*Phoenix dactylifera* L.) Fruit Growth and Development

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Keywords: metaxenia, date palm, fruit quality, hormonal changes

Abstract

This work was carried out in Balteem area, Egypt on the 'Hayany' date palm cultivar. Spaths were pollinated using either one of the two selected males (M_1 and M_2). Fruit samples were taken at different stages of its development to determine the changes in IAA, GA and ABA content.

Data indicated that there were obvious differences in 'Hayany' fruits that were produced from the two evaluated males. The concentration of the different growth regulators in consideration (IAA, GA, ABA) as well as the timing of their increase or decrease was also available between the two males. M_1 maintained a higher level of IAA than M_2 in most sampling dates. The highest IAA content coincided with rapid fruit growth.

Moreover, M_1 had higher GA content in most sampling dates especially in the 5th and 6th sampling date. This period coincided with the rapid growth phase. It was also shown that M_1 had a higher ABA content than fruit resulting from using M_2. This was clear especially in the 4-7th sampling date. A possible relationship between fruit growth and growth regulators content during fruit growth and development is suggested.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the leading fruit crops in Egypt. According to FAO statistics (2008) there are 87,685 hectares in Egypt, and the total production of date fruits amounts to 132,633 tons/year. Date palm fruits are known to have high nutritional value and are available at a reasonable price. This makes these fruits important for the consumer. Since date palm is a diocious plant, pollen has to be transported from the male to the female plant. This could occur naturally by wind, which needs rather a large number of male palms, or it can be carried out by man. This latter method is the method that is used where male palm is enough for pollinating about 25 female palms (Swingle and Nixon, 1928; Hussein et al., 1997). Thomas et al. (2000) studied the effect of different pollen parents on fruit set and development of lemon (*C. limon* Burn). Pollen source has an effect on fruit set, yield and fruit quality of some date palm cultivars (Osman et al., 1974; Iqbal and Ghafoor, 2000; Ghaffar and Iqbal, 2003; Iqbal, 2007). Also, a positive correlation between fruit retention and seed contents has already been reported (Dimri and Singh, 1991). They attributed differences in fruit growth to the young seeds number/fruit as they are the sites of cytokinin production. Auxin also prevents abscission. These explanations are in accordance with the results of Eti and Stosser (1992) in clementin mandarin and Caglar and Kasha (1995) in pistachio. Reuveni (1967) reported that four main promoters were found in the pericarps and seed of the fertilized date palm fruit during different phases of its development. However, none of these promoters was found to be closely correlated to fruit development. Thus total activity of promoters was calculated as IAA equivalent and indole aceto nitrite (IAN) activity. The highest level was found in the unfertilized fruits, the lowest in parthenocarpic triplets and intermediate in parthenocarpic single fruits. Evidence was

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found that the high levels found in fertilized fruits originated from seed. Positive correlation between fruit set, gain in weight, and growth promoting activity was found during the 1st period of growth till the end of the kimiri stage. The requirement for growth regulators for growth was low in the beginning, high at the stage of rapid growth, and low close to fruit maturation.

The IAA content of 'Hellawi' date palm in Iraq was very high in non-pollinated flowers, then declined at fruit set, rose again as the fruits entered the rapid phase of growth, then declined as fruits advanced toward the ripening phase, and the changes in IAA concentration during fruit development may reflect the role of this natural hormone in the control of various fruit developmental stages (Abbas et al., 2000). In fruits of low chilling pear cultivars, ABA content in the developing fruits in all the three cultivars was lower in all periods compared with IAA. However, both regulators have a similar trend, being higher in the initial stage of fruits development, decrease to a lower level at 50 and 80 DAFB and then increasing slightly as the fruit approached maturity. They noted varietal differences in IAA as well as some unidentified compounds (Dwivedi and Bist, 2002).

Balteem district, Kafer El-Sheikh Governorate, Egypt is considered one of the intensive date palm growing districts in Egypt. There are about 300,000 female date palms in this district. The work indicated in this work was carried on 'Hayany' date palm growing in Balteem area where the following parameters were investigated:

1. Two superior males were used out of these 20 evaluated males to study their subsequent effect on fruit physical and chemical characters. Moreover, fruit set was also determined.
2. Fruit samples were taken at different stages of fruit development to determine the changes in IAA, GA and ABA as related to different used males during fruit development as a trial to explain the metaxenic effect.

MATERIAL AND METHODS

This investigation was carried out during the 1999 and 2000 seasons in Balteem district, Kafr El-Sheikh Governorate, Egypt. Two males ('Meghal') were used for pollinating 'Hayany' date palms to study the effect of pollen source on fruit set (%), bunch weight and changes in fruit characters at different intervals after fruit set and their IAA, GA and ABA content were determined.

Effect of Selected Males on 'Hayany' Fruit Characters

Three healthy, 11-year-old 'Hayany' female date palms were selected for each male, 9 bunches for each female were selected. The palms were grown in loamy soil and planted 8 meters apart, and receiving the regular horticultural practices recommended by the Ministry of Agriculture. Pollinated bunches (9 strands/bunch) were bagged with paper bags 15 days after pollination date.

Some Endogenous Hormones Determination

'Hayany' fruit samples (9 samples) were taken (about 5 g) after 3, 6, 9, 16, 23, 30, 44, 58 and 72 days after pollination date (30 Mar., 2 Feb., 6 Feb., 14 Feb., 21 Feb., 28 Feb., 12 Apr., 26 Apr. and 9 Jun). These samples were representatives of the two males used in the metaxenic studies. The samples were frozen immediately on dry ice, and stored at -20°C until the time of extraction.

The analysis of these samples was carried out at the Arid Land Agricultural Research Unit, Faculty of Agriculture, Ain-Shams University, Egypt where the method described by Shindy and Smith (1975) was used.

Fruit Set Percentage

The number of fruits retained on the strand and the total number of flowers scars was counted and fruit set was determined as recommended by El-Makhtoun (1981). Fruit set was calculated every two weeks (during May and June) using the following equation:

$$\text{Fruit set (\%)} = \frac{\text{No. of fruits retained on strand}}{\text{No. of fruits retained + No. of flower scars on the same strand}} \times 100$$

Determinations at Harvest

All trees were harvested at the peak of color development at the first week of October (Khelal stage). The samples contained 30 representative fruits randomly picked for each treatment in order to determine the following:

1. Physical fruit characteristics: 15 fruits/treatments were taken to determine fruit length and diameter (cm), fruit and flesh weight (g), volume (cm^3).
2. Chemical fruit characteristics: fruit firmness (kg/mm^2) was determined by (A.O.A.C., 1989). Tannins content (%): samples of fruits were taken to determine tannins content as "galacturonic" acid and calculated as mg/100 g fresh weight (A.O.A.C., 1989). Total soluble solids (TSS): TSS was determined by RL3 Refractometer (A.O.A.C., 1989) in the juice extracted from 10 fruits. Vitamin C was determined and calculated as mg/100 g flesh weight according to (A.O.A.C., 1989). Sugars content: total and reducing sugars were determined by colorimetric methods recommended by Forsee (1938).

Statistical Analysis

All data were statistically analyzed according to Randomized Complete Block Design (RCBD)-significance of differences among treatments means were judged using Duncan Multiple Range Test (DMRT, according to Snedecor and Cochran, 1977).

RESULTS AND DISCUSSION

Endogenous Growth Hormones during Fruit Growth and Development

Figures 1-8 indicated that there were obvious differences in 'Hayany' fruits that were produced from the two pollen sources used. The concentration of different growth regulators that were determined (IAA, GA and ABA), as well as the timing of their increase or decrease was also variable between the two males. Male 1 (M_1) had generally higher IAA content than M_2. It was noticed that there was an increase in IAA content in the early part of fruit development in both males. However, this surge was somewhat at a later date in M_2 than in M_1. Thereafter the concentration of IAA in both males decreased, however, with M_1 maintaining higher level of this growth regulator than M_2 in most sampling dates. Figures 1 and 2 show the rate of fruit growth during the sampling dates, and their IAA content in the two studied males. It could be seen that the highest IAA content coincided with rapid fruit growth. The best-fit curve indicated that there was a decreasing trend thereafter. Most important is the observation that higher IAA concentration in M_1 was associated with larger fruit weight as compared with M_2, which might indicate in part the reason for differences that were observed in fruit weight.

From Figures 3 and 4 it is evident that M_1 had a higher GA content in most sampling dates especially on the 5^{th} and 6^{th} sampling dates. This period coincides with the rapid growth phase. Again, it is not surprising that M_1 had larger fruits than M_2. When fruit GA content was plotted with fruit development with time, it became evident that GA generally increased gradually till the 5^{th} sampling date, and then dropped again. This became rather clear when drawing the best-fit curve.

It could also be seen from Figures 5 and 6 that the ABA content of fruits of 'Hayany' date palm, at different stages of fruit development showed that M_1 pollinated fruits generally had a higher content than M_2 fruits.

In both males, the ABA content of the produced fruits increased with the highest rate of fruit growth. This coincided with the 4^{th} to 7^{th} sampling date (April 14^{th} to May 12^{th}). Thereafter, the ABA content decreased generally as indicated from the best-fit curve.

Effect of Male Types on

1. Fruit Set (%). Data indicated that the pollen source affected the fruit set percentage of 'Hayany' date palm in both seasons of this study. Male 2 had a higher fruit set % value (26.02%) as compared with (23.33%) in male No. 1 [M_1] in both seasons, respectively (Fig. 7).

These results are in agreement with those obtained by those who reported that a different pollen source resulted in a different fruit set percentage and these effects differed between years and between the male or female cultivars (Marzouk et al., 2002a, b; Ghaffar and Iqbal, 2003; Iqbal, 2007).

2. Bunch Weight (kg). Bunch weight is considered as an index for the yield and reflects the best promising treatments used in the present investigation. Data illustrated show statistically significant differences in the first season. The highest bunch was obtained when pollen from $M_1$was used in both seasons (Fig. 7).

These results are in agreement with those reported by El-Kassas et al. (1996), El-Salhy et al. (1997), Marzouk et al. (2002a, b), Helail et al. (2001), Ghaffar and Iqbal (2003) and Iqbal (2007). They reported that there was a positive correlation between fruit set percentage and bunch weight obtained at harvest.

3. Physical Properties. Data indicated the effect of different date pollen grains on the changes in physical fruit properties of the 'Hayany' cultivar in both seasons. It is obvious that the 1st male caused an increase for most studied fruit physical characters in comparison with values obtained for M_2 as indicated in the following:

Fruit Weight (g). Fruit weight of 'Hayany' palms grown in Balteem was significantly affected by pollen source, where M_1 had higher values than M_2. However, the differences were insignificant in both seasons (Iqbal and Ghafoor, 2000; Marzouk et al., 2002a, b; Ghaffar and Iqbal, 2003; Iqbal, 2007).

Flesh Weight (g). Pollination with different pollen types significantly affected flesh weight only in the first season, where M_2 had higher flesh weight than M_1. These results partially agree with Marzouk et al. (2002a, b) and Helail et al. (2001), who found that pollen source has a significant effect on flesh weight of date fruits (Fig. 7).

Fruit Length (cm). The two used males on 'Hayany' fruits did not significantly affect the fruit length in both seasons. However, the data indicated that M_2 gave generally higher values than M_1 (Fig. 7). These finding are in harmony with those reported by Schroeder and Nixon (1958), El-Salhy et al. (1997), Rahemi (1998), Iqbal and Ghafoor (2000), Ghaffar and Iqbal (2003) and Iqbal (2007) who indicated that specific pollens may possibly affect the cell number in early fruit development.

Fruit Diameter (cm). It is clear from such that pollen grains of M_2 recorded a significantly higher diameter in the second season than M_1. These results agree with El-Salhy et al. (1997), Aly (2001) and Helail et al. (2001) who found that pollens source significantly affected fruit length and diameter.

Fruit Volume (cm^3). Pollen source had no significant effect on fruit volume of 'Hayany' palms grown in Balteem in the two seasons at fruit harvest (Fig. 7). These data are in agreement with those obtained by Nixon (1926, 1927, 1965), Abdelal et al. (1993), Hussein et al. (1999) and Aly (2001).

Fruit Firmness (kg/mm^2). Concerning the effects of different pollen types on fruit firmness, it was shown that the lowest values of fruit firmness are found in 'Hayany' females pollinated with M_2. The differences between fruit firmness of M_1 and M_2 were significant in both seasons. This might indicate earlier maturation or crisper pulp. Similar findings were reported by Nixon (1965), Ream (1976) and Marzouk et al. (2002a, 2002b).

4. Chemical Properties. It could be observed that the two different pollen grains were variably different in their effect. In all cases the values obtained in the second season were higher than those obtained from the first season, which may be due to the fluctuation in the environmental conditions during the two seasons (Fig. 8).

TSS (%). Significant differences were found in the TSS (%) content of 'Hayany' fruits due to the pollen source in the two seasons. These data contradict those obtained by Maradi (1995), El-Kassas et al. (1996), Iqbal and Ghafoor (2000), Marzouk et al. (2002a,

2002b), Ghaffar and Iqbal (2003) and Iqbal (2007) who reported that total soluble solids seemed to be influenced by the type of used pollen (Fig. 8).

Tannins (%). It is quite clear that in both seasons 'Hayany' date palms pollinated with two different pollen sources show insignificant different tannin content in the two experimental seasons (Maradi, 1995; El-Kassas et al., 1996; Marzouk et al., 2002a, 2002b).

Vitamin C. Statistical analysis showed no significant differences in both seasons, concerning tannin content of the fruits. The range of vitamin C content reported here is in agreement partially with those values reported by Sawaya et al. (1983) who found that the range of vitamin C varies from 4.9 to 9.0 mg/100 g flesh weight during kimri to rutab stages for the 'Khudari', 'Sillaj' and 'Sifri' cultivars pollinated by the same pollen (Marzouk et al., 2002a, b) (Fig. 8).

Total Sugars (%). Pollination treatments affected total sugar percentages insignificantly through the two studied seasons. As such, the highest average of total sugars (25.04%) was recorded in the second season in 'Hayany' dates that were pollinated with the first pollen source and the lowest (24.52%) in the first season in 'Hayany' dates that were pollinated with the second pollen source. Generally, pollination treatments by the first male (M_1) pollen produced fruits with higher values in total sugars (Fig. 8).

These results were not in agreement with El-Kassas et al. (1996), Iqbal and Ghafoor (2000), Marzouk et al. (2002a, b), Ghaffar and Iqbal (2003) and Iqbal (2007) who found that the male type had a significant effect on total sugars content.

Reducing Sugars(%). In both years the first male recorded the highest average (17.32%) as compared with the results recorded for the second male (16.65%) (Fig. 8).

These results are in line with El-Kassas et al. (1996), Marzouk et al. (2002a, b), Ghaffar and Iqbal (2003), and Iqbal (2007) who found that reducing sugars content of date fruits depends on the type of pollen used.

CONCLUSIONS

The two males had different effects on the physical as well as the chemical fruit characters on 'Hayany'. Moreover, the ripening date was also affected. Similar findings were reported by several workers (Hussein and El-Dosouky, 1992; Abdella and Abou-Sayed Ahmed, 1993; Abo-Sayed-Ahmed, 1993; El-Makhtoun et al., 1994, 1995; El-Kassas et al., 1996; Abdel-Hamid, 2000; Aly, 2001; Helail et al., 2001; Hussein and Hassan, 2001; Marzouk et al., 2002a, b).

Data of Reuveni (1967) indicated that high levels of promoters were found in the fertilized fruits. However, no comparison was made between different males in this study. Moreover, paper chromatography was used in this work, which is less efficient than the present available techniques.

The present work indicated that there was an increase in IAA, GA and ABA in the first growing phase of 'Hayany' date palm fruits that were fertilized by either studied male palm. M_1 was found to have a higher hormonal content than M_2. This was related to fruit growth. These hormones were shown to be essential for fruit development (Buta and Spalding, 1994; Ben Cheikh et al., 1997; Rahemi, 1998; Iqbal and Ghafoor, 2000; Ghaffar and Iqbal, 2003; Iqbal, 2007). The importance of pollen source on fruit set and development was also indicated for clementine (Eti and Stosser, 1992), pistachio (Cagler and Kasha, 1995), and lemon (*C. limon*, Burn) (Thomas et al., 2000).

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Figures

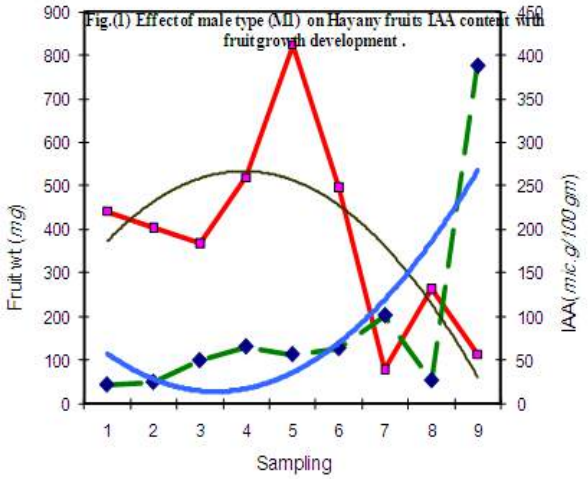

Fig. 1. Effect of male type (M_1) on 'Hayany' fruits IAA content with fruit growth develpoment.

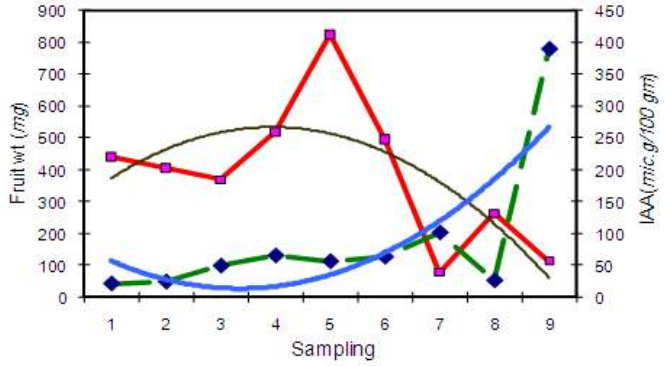

Fig. 2. Effect of male type (M_2) on 'Hayany fruits IAA content with fruit groth development.

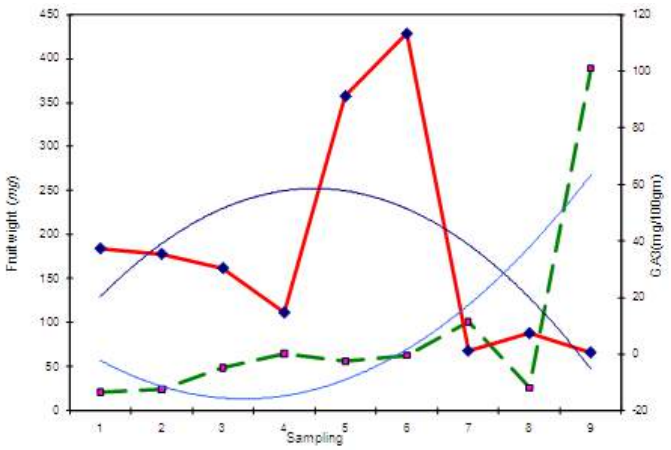

Fig. 3. Effect of male type (M_1) on 'Hayany' fruit GA_3 content with fruit growth development.

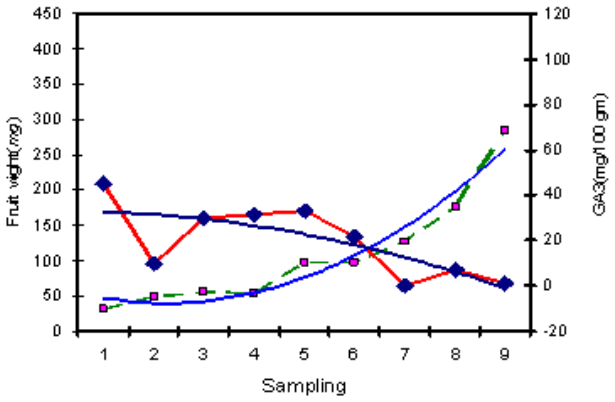

Fig. 4. Effect of male type (M_2) on 'Hayany' fruits GA_3 content with fruit growth development.

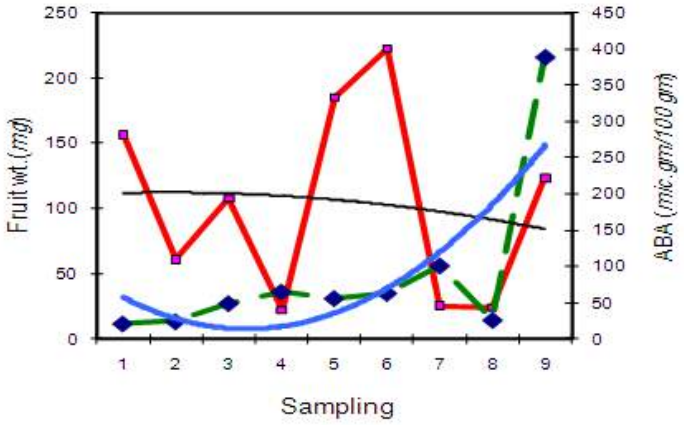

Fig. 5. Effect of male type (M_1) on 'Hayany' fruits ABA content with fruit growth develoment.

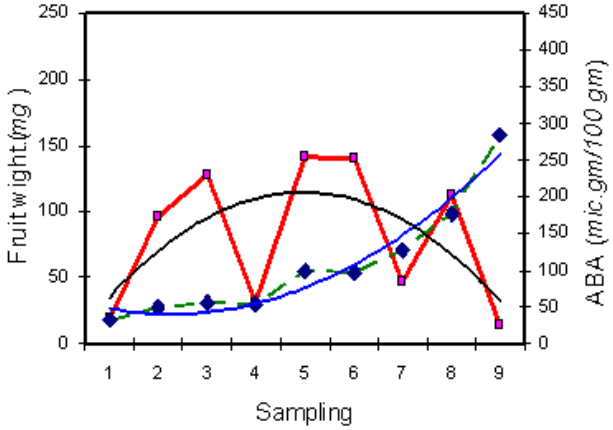

Fig. 6. Effect of male type (M_2) on 'Hayany' fruits ABA content with fruit growth development.

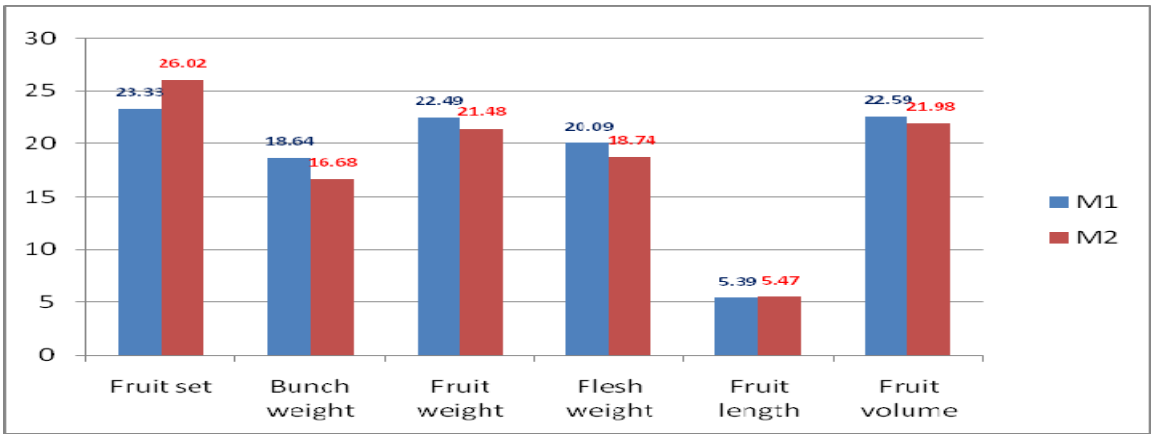

Fig. 7. Effect of male types on physical characteristics of 'Hayani' fruit.

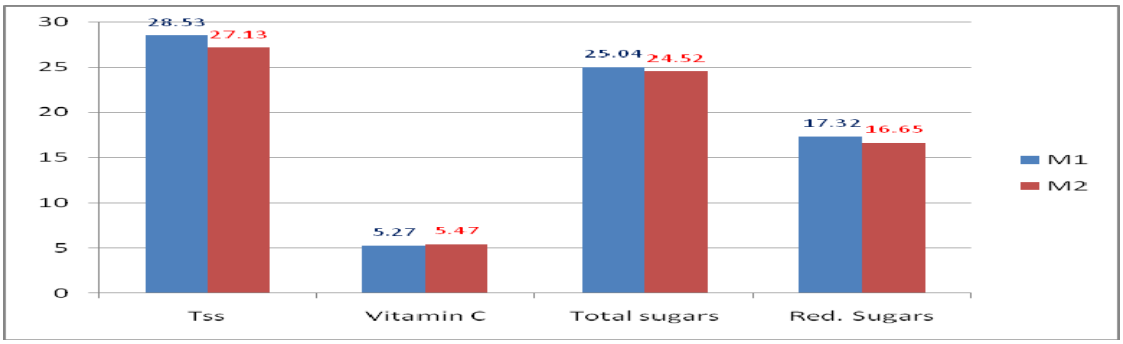

Fig. 8. Effect of male types on chemical characteristics of 'Hayani' fruit.

An Efficient Novel Pathway Discovered in Date Palm Micropropagation

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Keywords: *Phoenix dactylifera* L., in vitro, direct embryogenesis, polyamines (PAs)

Abstract

Direct somatic embryos of date palm (*Phoenix dactylifera* L. 'Medjool') spontaneously developed on the individually proliferated axillary buds. Axillary bud proliferation occurred from shoot tips explants under dark condition afters 3 recultures on modified MS (Murashige and Skoog, 1962) medium supplemented with 2iP (1.0 mg/L), Kin (1.0 mg/L), BA (1.0 mg/L), NOA (0.5 mg/L) and solidified with gelrite (2.0 g/L). When buds were transferred under light conditions onto the same medium containing putrescine (150 mg/L), some of them (55.56%) showed direct embryos formation (3.78) at the surface of the buds after 8 weeks of culture incubation. When these embryos were soon removed after they became visible, they survived by transferring to a fresh medium, whereas they were destroyed if left intact with the bud. Embryos cultured on the previous medium omitting BA in addition to putrescine (100 mg/L) recorded the highest significant values of multiplication rate and growth value after 6 weeks of incubation. Individual shoots cultured on basal MS medium in addition to IBA (1.0 mg/L), sucrose (30 g/L) and solidified with phyto-agar (6.0 g/L) recorded the highest significant values of roots number and roots length after 8 weeks of incubation. Finally, using a mixture containing compost and perlite (1:1, v/v) recorded the highest significant percentage values of plantlets survival (80%) and number of leaves/plantlet (3.67) as well as the highest values of leaf length (17.1 cm) after 3 months in acclimatization. Phenotypically, plantlets showed similarity in the greenhouse.

INTRODUCTION

Date Palm (*Phoenix dactylifera* L.) is dioecious perennial species (2*n*=36) of the *Arecaceae* and is one of the most important *Liliopsidaous* fruit crops in Arab countries. The family *Arecaceae* comprises about 200 genera and 2500 species distributed through tropical and subtropical regions around the world (FAO statistics, 2008). Date palm propagate either by seeds but the resulted seedlings differ considerably in fruit quality, harvesting time and production potential or by offshoots, but this method of propagation has been faced by the fact that date palm produce relatively few offshoots suitable for transplanting in its lifetime (Zaid and De Wet, 2002). Rapid propagation of date palm through tissue culture is the most promising technique for production of sufficient plant materials with high quality (Sane et al., 2006). In reviewing date palm micropropagation, several reports have been published. The early attempts via zygotic embryos have been done by Schroeder (1970). However, various explants tissues have been examined i.e., shoot tip (El-Bellaj et al., 2000; Al-Khayri, 2001), leaf primordial (Beauchesne et al., 1986; Ibrahim and Hegazy, 1998, 1999, 2001; Hegazy et al., 2009), axillary bud (Sharma et al., 1984; Zaid and Tisserat, 1983a), root (Zaid and Tisserat, 1983b) and finally inflorescence (Drira and Benbadis, 1985; Hegazy, 2008). Organogenesis and somatic embryogenesis are the two techniques currently used in various laboratories in the world for in vitro mass propagation of date palm (Al Kaabi et al., 2007). Organogenesis in date palm has a low efficiency due to the low number of explants that respond in vitro, the

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long time required for the initiation phase, the low multiplication rate and the strong influence of the variety (Beauchesne et al., 1986). Somatic embryos could be formed indirectly through callus (Tisserat, 1984) or directly without any intervening callus phase (Hegazy, 2003). In palms, until 40 years ago, the most reports were achieved through indirect somatic embryogenesis pathway. Only few workers attempted the direct organogenesis method (Ibrahim and Hegazy, 1999; Drira and Benbadis, 1985; McCubbin and Zaid, 2007) and the direct embryogenesis method (Hegazy, 2003).

The objective of this work was to study the availability of micropropagating the high quality date palm ''Medjool'' (semidry cultivar 20-30% moisture content in their fruits) through a pioneer pathway (direct somatic embryos occurred on the proliferated axillary buds), to avoid callus regeneration off-types is a rather new approach.

MATERIALS AND METHODS

The present investigation was carried out in the Plant Tissue Culture Laboratory, Plant Biotechnology Department of the Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University Egypt, during the period 2007-2010. Shoot tips explants were obtained from 3-year-old offshoots of the female date palm 'Medjool' having high quality fruits grown at Mr. Nabil Abdrabo's model farm, Al-Katta Desert, Gizza governorate, Egypt.

The MS basal medium (Murashige and Skooge, 1962) modified with [glutamine (200 mg/L), bioten (0.5 mg/L), adenine sulphate (20 mg/L), thiamine-HCl (10 mg/L), Ca-pantothenate (10 mg/L), ascorbic acid (75 mg/L), citric acid (75 mg/L), NaH_2PO_4. $2H_2O$ (170 mg/L), activated charcoal (1.5 g/L) and raised sucrose up to 40 g/L]was used. The pH of the media was adjusted to 5.6 with 0.1 M KOH or 0.1 M HCl prior to gelling agent addition. Media were dispensed either in a glass tubes (25×2.5 cm; Borosil) capped with Bellco plastic caps containing 15 ml or into jars (150 ml) at the rate of 35-40 ml/jar and autoclaved at 121°C and 1.2 kg/cm^2 for 20 min.

Plant Material Sterilization

For excising the shoot tip explant, the outer leaves and fiber sheath were removed from offshoots acropetally with a hatchet and sharp knife (always sprayed with etheyl alcohol 70%) until they reached 3.0-4.0 cm in width and 6.0-8.0 cm in length. Under aseptic conditions, plant materials were surface sterilized twice by soaking in a commercial disinfectant Clorox (5.25% NaOCl) solution 2% for 30 min, 2 drops/100 ml solution of Tween 20 (polyoxyethylenesorbitan monolaurate) as wetting agent were used. Then, they were rinsed with sterile distilled water followed by being immersed in sterilized mercuric chloride solution (0.1%) for 10 min. They were then rinsed with sterile distilled water and soaked in a filter sterilized antioxidant solution (citric acid and ascorbic acid) each at a concentration of 150 mg/L. Finally they were kept in the refrigerator (8°C) for 2 hours prior to shortening. Additional leaves were removed one by one till shoot tip was reached (0.7 to 1 cm^2), then cultured.

1. Effect of Some Growth Regulators on Axillary Bud Proliferation. Shoot tips were cultured individually on modified MS medium supplemented with auxin [NOA (naphthoxy acetic acid)] and cytokinins [BA (6-benzylaminopurine), Kin (6-Furfurylaminopurine) and 2iP (6-(ŷ,ŷ-dimethylallylamino purine)] were used (mg/L); control (free hormone), NOA (0.5), {NOA: BA, 0.5:1}, {NOA: Kin, 0.5:1}, {NOA: 2iP, 0.5:1} and {NOA: BA: Kin: 2iP, 0.5:1:1:1} and solidified with gelrite (2 g/L). Nine jars (replicates) were used for each treatment. Cultures were incubated in total darkness at 25±1°C and recultured 3 times to the same medium with 4-week intervals. Data of fresh weight (g) and axillary bud proliferation % of the three consecutive recultures were recorded.

2. Effect of Putrescine on Embryos Formation. Proliferated axillary buds obtained from the previous experiment were carefully separated from shoot tips and individually cultured on the best growth regulators combination of the previous experiment in addition to polyamine (PAs) i.e., putrescine at concentrations of 0, 100, 150 and 200 mg/L. Nine

jars (replicates) were used for each treatment. Cultures were exposed to 16-h photoperiod using fluorescent tubes with a light intensity of 1500 lux for 8 weeks. Each 4 weeks, data of bud formed embryos % and number of embryos/bud were recorded.

3. Effect of Embryos Position. To overcome the obstacles facing somatic embryos development axillary buds originating from embryos resulting from the previous experiment were transferred to the same medium and their embryos were subjected to two positions: 1) embryos removal from the bud, 2) embryos left intact with the bud. Nine jars were used for each treatment. Incubation was achieved at the same previously mentioned conditions. After 8 weeks, percentages of embryo survival and growth were recorded.

4. Effect of Putrescine on Embryos Multiplication Rate and Growth Value. Previously resulted embryos were cultured on the axillary bud proliferation medium omitted BA in addition to putrescine at the concentration of 0, 50, 100 and 150 mg/L. Nine jars (replicates) were used for each treatment. Incubation was carried out under the same embryos formation conditions. After 6 weeks, data of embryos numbers, multiplication rate, fresh weight (g) and growth value were recorded.

5. Effect of IBA on Root Formation. Healthy shoots were individually cultured on basal MS medium supplemented with sucrose (30 g/L) in addition to IBA (indole-3-butyric acid) at the concentration of 0, 0.5, 1.0 and 2.0 mg/L and solidified with phyto-agar (6.0 g/L). Nine glass tubes (replicates) were used for each treatment. Cultures were incubated under the same conditions previously mentioned with raised light intensity up to 3000 lux. After 8 weeks, data of root formation %, number of roots and root length (cm) were recorded.

6. Effect of Soil Mixture Types on Acclimatization. Plantlets were removed from the rooting medium, rinsed under tap water and immersed in water for 2h. Roots were immersed in a fungicide (Benomyl) solution (0.5%, w/v) for 5 min. Then, they were planted in plastic pots (5×18 cm) filled with a soil mixture as follows: compost and sand (1:1, v/v), compost and peat moss (1:1, v/v), compost and perlite (1:1, v/v) and compost and vermiculite (1:1, v/v). The plants were covered with transparent polyethylene sheet and sub-irrigated if needed. The potted plants were incubated for 30 days in phytotron at 25±1ºC, relative humidity (80-90%) and 16-h photoperiod with a light intensity of 1500 lux. Acclimatization was achieved through gradually removing the plastic sheet each day till it was totally removed after 30 days. Plants were transferred to a plastic greenhouse in tunnel under shade condition (black saran 63%) and were left to grow for another two months. Plants were sub-fertigated once a week with commercial fertilizer of NPK (Sangral, 1.0 g/L) at a ratio of 20:20:20. After 3 months survival %, number of leaves/plant and leaf length (cm) were recorded.

7. Growth Value. Embryos' growth values were estimated according to the equation of Ziv (1992). $GV = \frac{Fw_F - Fw_i}{Fw_i}$ where, GV = Growth value. Fw_F = Final fresh wt. Fw_i = Initial fresh wt.

8. Statistical Analysis. Data were statistically analyzed by one factorial randomized complete design using the SAS (1988) package. The Least Significant Differences among levels of each treatment were compared using LSD test at 5%, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

As a result of reviewing a large numbers of date palm micropropagation studies, which demonstrated the in vitro pathways and morphogenesis obtained from different date palm explants (Fig. 1) it is predicted that this is a pioneer report on initiating direct somatic embryos on proliferated axillary buds.

Effect of Some Growth Regulators on Axillary Bud Proliferation

Results in Table 1 and Figures 2A and B showed that shoot tips recultured 3 times on modified MS medium supplemented with NOA (0.5 mg/L) in combination with an

equal level of BA (1.0 mg/L), kin (1.0 mg/L) and 2iP (1.0 mg/L) recorded the highest significant values of fresh weight 4.09, 8.06 and 15.06 g in the three consecutive recultures respectively. This was accompanied by higher significant percentage values of axillary bud proliferation (44.44%) as compared with the other studied treatments. In this regard, Beauchesne et al. (1986) found that, at the bottom of the young leaves some very little axillary buds are often visible. Auxins at low concentration, enhanced date palm bud growth in vitro after four to six months, gave some signs of budding which indicates giving true-to-type plantlets. Ziv (1991) stated that in vitro explant culturing necessitates a continuous supply of growth regulators to the culture medium i.e., auxins and cytokinins supplied either singly or in combination at diverse ratios, depending on the species and the type of explant. Date palm shoot tips cultured on medium containing low auxin concentrations initiated leaves and in some cases roots while, high auxin concentrations resulted in the formation of callus (Tisserat, 1979). Also, Mater (1986) reported that high auxin levels in the medium favored callus growth and low auxin levels favored normal vegetative growth of date palm shoot tips. On the other hand, Zaid and Tisserat (1983a) reported that addition of growth regulators to the nutrient medium was not necessary to stimulate shoot proliferation, better shoot tip development occurred on 10 and 100 mg/L NAA. In addition, Tisserat (1984) reported that addition of cytokinin at any level to date palm tissue culture media did not enhance shoot differentiation.

Effect of Putrescine on Embryos Formation

Data presented in Table 2 and Figures 1C, D, 2C and D showed that addition of putrescine to the axillary bud proliferation medium at the concentration of 150 mg/L recorded the highest significant percentage values of bud formed embryos (55.56%) and number of embryos/bud (3.78) after 8 weeks as compared with the other studied concentration treatments, i.e., 0, 100 and 200 mg/L. However, among all putrescine concentrations treatments in the first 4 weeks incubation, only addition of 200 mg/L recorded bud formed embryos (11.11%) and number of embryos/bud (0.67). In contrast, those formed embryos encountered a problem in growth, turned brown and were destroyed after 8 weeks. Here, it is hypothesized that this might be as the consequence of embryos left intact with the bud more than 4 weeks caused profound alterations in developmental processes. Altogether this information will be valuable for ongoing experiments. Srivastava (2002) published that polyamines (PAs) are generally recognized as active regulators of plant growth. They are present in all cells, and their mMolar titer is responsive to physiological effects caused by many agents, such as hormones, light and stress, but their precise mode of action in plant growth and development is still unclear.

Effect of Embryos Position

Data presented in Table 3 and Figure 2E indicates that embryo removal from the bud recorded enormous significant percentages values of survival and growth (100%) after 8 weeks of incubation as compared with embryos left intact with the bud (0%). With regard to the undesirable distinctly destroyed embryos resulted, they might not be in a proper position which retards their development and should be discouraged prior to a certain waste. In this concern Racusen and Schiavone (1990) reported that concerning the origin and perception of positional information in plant embryos and the temporal spatial expression of genes. The spatial distribution of certain gene products is correlated with changes in morphology. However, there is, as yet, insufficient evidence with which to forge a link between positional cues and the expression of genes which influence developmental transitions in embryos. In addition, Hegazy (2003) reported that controlling of date palm embryo's hyperhydricity was achieved by placed embryo clusters submerged in culture medium.

Effect of Putrescine on Embryos Multiplication Rate and Growth Value

Results presented in Table 4 and Figures 2F and G illustrate that embryos cultured on the axillary bud proliferation medium omitted BA in addition to putrescine (100 mg/L)

scored the highest significant values of multiplication rate and growth value after 6 weeks of incubation as compared with the other studied concentration treatments, i.e., 0, 50 and 150 mg/L. In contrast, elevated putrescine concentration up to 150 mg/L recorded a significant reversible effect in all growth characters as compared with the best treatment 100 mg/L. Similar results were obtained by Hegazy (2008) who found on date palm floral buds 'Selmy' that embryos cultured on modified MS medium in addition to putrescine (100 mg/L) obtained significant values of multiplication rate and growth value as well as total soluble protein and phenylalanine ammonialyase (PAL) activity. Srivastava (2002) published that PAs metabolism is affected by auxins, cytokinins, and gibberellins in several plant systems and that PAs are essential for many of the growth responses attributed to these hormones. The specific roles of PAs in these responses are unknown.

Effect of IBA on Root Formation

Data presented in Table 6 and Figure 2H indicate that, shoots cultured on basal MS medium supplemented with IBA (1.0 mg/L) recorded the highest significant values of root formation (100 %), roots number (3.11) and root length (3.88 cm) as compared to the other studied concentrations. Srivastava (2002) published that IBA is known as a major synthetic auxin used commercially for the induction of adventitious roots. Recently, it has also been identified as a naturally occurring compound derived from IAA via a chain elongation reaction. IBA is also converted to IAA. Reasons for the greater potency of IBA are unclear. Genetic analysis further indicated that high and low rooting responses were probably controlled by multiple genes. In addition, Sane et al. (2006) on date palm reported that rooting without hormone resulted in the development of fine ramified roots that were unable to survive when planted in a nursery. However, Picoli et al. (2001) mentioned that failure of hyperhydric plants to grow when transferred to soil may often be due to malfunctioning of the leaf rather than the poor rootability.

Effect of Soil Mixture Types on Acclimatization

Data presented in Table 6 and Figure 2I show that soil mixture containing compost and perlite (1:1, v/v) recorded the highest significant percentage values of plantlets survival (80%) as well as higher leaves number (3.67) and leaf length (17.1 cm) as compared with the other studied soil mixture types. However, compost and sand (1:1, v/v) recorded the lowest results in plantlets survival (23.33), leaves number (3.11) and leaf length (10 cm). In this concern, (Hegazy, 2008) suggested that the superiority of compost and perlite could be ascribed to their effects on sparring more suitable conditions for growing roots. Compost might increase the organic matter content, which in turn improved the soil physical condition. Perlite could hold three to four times its weight of water as well as it was most useful in increasing aeration in mixture. Phillips and Hubstenberger (1995) reported that repetitive embryogenesis showed the best balance of high propagation rates with relatively few off-types. Hegazy (2003) reported that direct embryogenesis avoids the genetic instability often associated with somatic embryos obtained indirectly. The high similarity index detected using AFLP as a molecular procedure for DNA fingerprinting for 'Zaghloul' and 'Barhee' vitroplants with their corresponding mothers might indicate that the genetic makeup of the mothers has been retained and therefore, the derived plantlets are true-to-type.

Future Work

We hoped to continue these studies to confirm the obtained vitroplants' similarity at the molecular level and to evaluate the productivity with regard to the mother.

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Tables

Table1. Effect of plant growth regulators on fresh wt. (g) and auxiliary bud proliferation of date palm shoot tips 'Medjool' cultured in vitro for 12 weeks.

Treatment (mg/L)		Growth characters				
		Initial fresh wt. (g)	Final fresh wt. (g) Recultures			Auxiliary bud proliferation (%)
			1	2	3	
Free hormone		0.97[a]	1.72[f]	2.11[f]	2.54[f]	00.00[b]
NOA (0.5)	+------	0.89[b]	2.29[e]	3.43[e]	3.69[e]	00.00[b]
	+BA (1)	0.92[b]	3.78[b]	6.58[b]	13.18[b]	00.00[b]
	+Kin (1)	0.93[ab]	2.80[d]	5.04[d]	8.76[d]	00.00[b]
	+2iP (1)	0.91[b]	3.07[c]	6.01[c]	9.95[c]	00.00[b]
	+BA (1)+Kin (1)+2 iP (1)	0.94[ab]	4.09[a]	8.06[a]	15.06[a]	44.44[a]

Means within each column followed by the same letter are not significantly different at P=0.05.

Table 2. Effect of putrescine concentrations on bud formed embryos and number of embryos/bud of proliferated date palm axillary buds 'Medjool' cultured in vitro for 8 weeks.

Treatment (mg/L)		Growth characters			
		4 weeks		8 weeks	
		Bud formed embryos (%)	Number of embryos/bud	Bud formed embryos (%)	Number of embryos/bud
Putrescine	0	00.00[a]	00.00[a]	00.00[a]	00.00[a]
	100	00.00[a]	00.00[a]	00.00[a]	00.00[a]
	150	00.00[a]	00.00[a]	55.56[a]	3.78[a]
	200	11.11[a]	0.67[a]	11.11[b]	0.67[b]

Means within each column followed by the same letter are not significantly different at P= 0.05.
***** Embryos turn brown and are destroyed because they may be left intact with the bud.

Table 3. Effect of embryos position either removal or left intact with the axillary bud of date palm ‘Medjool’ cultured in vitro for 8 weeks.

Position treatment	Growth characters	
	Embryos survival	% of growth
1. Embryos intact with the bud	0[b]	0[b]
2. Embryos removal from the bud	100[a]	100[a]

Means within each column followed by the same letter are not significantly different at P=0.05.

Table 4. Effects of putrescine on embryos multiplication rate and growth value of date palm ‘Medjool’ embryos cultured in vitro for 6 weeks.

Treatment (mg/L)		Growth characters					
		No. of embryos		Multiplication rate	Embryos f. wt. (g)		Growth value
		Started	Produced		Started	Produced	
Putrescine	0	5.87[a]	13.00[c]	2.34[d]	0.57[a]	1.90[c]	2.35[c]
	50	6.00[a]	14.89[bc]	2.48[c]	0.63[a]	2.65[b]	3.36[b]
	100	5.78[a]	18.11[a]	3.13[a]	0.54[a]	3.32[a]	5.32[a]
	150	6.11[a]	16.78[ab]	2.73[b]	0.59[a]	2.89[b]	3.90[b]

Table 5. Effects of IBA concentrations on root formation of date palm ‘Medjool’ shoots cultured in vitro for 8 weeks.

Treatment (mg/L)		Root growth characters		
		Formation (%)	Number	Length (cm)
IBA	0.0	11.11c	0.22c	0.28c
	0.5	33.33bc	0.56bc	0.86c
	1.0	100.00a	3.11a	3.88a
	2.0	55.56b	1.33b	2.24b

Means within each column followed by the same letter are not significantly different at P=0.05.

Table 6. Effect of soil types on survival percentage, number of leaves and leaf length of ‘Medjool’ plantlets after 3 months in acclimatization.

Treatments			Growth characters		
			Survival (%)	Leaves	
				Number	Length (cm)
Compost	+Sand	(1:1, v/v)	23.33[c]	3.11[b]	10.0[c]
	+Peatmoss	(1:1, v/v)	43.33[bc]	3.33[ab]	12.6[b]
	+Perlite	(1:1, v/v)	80.00[a]	3.67[a]	17.1[a]
	+Vermiculite	(1:1, v/v)	50.00[b]	3.22[ab]	15.7[a]

Figures

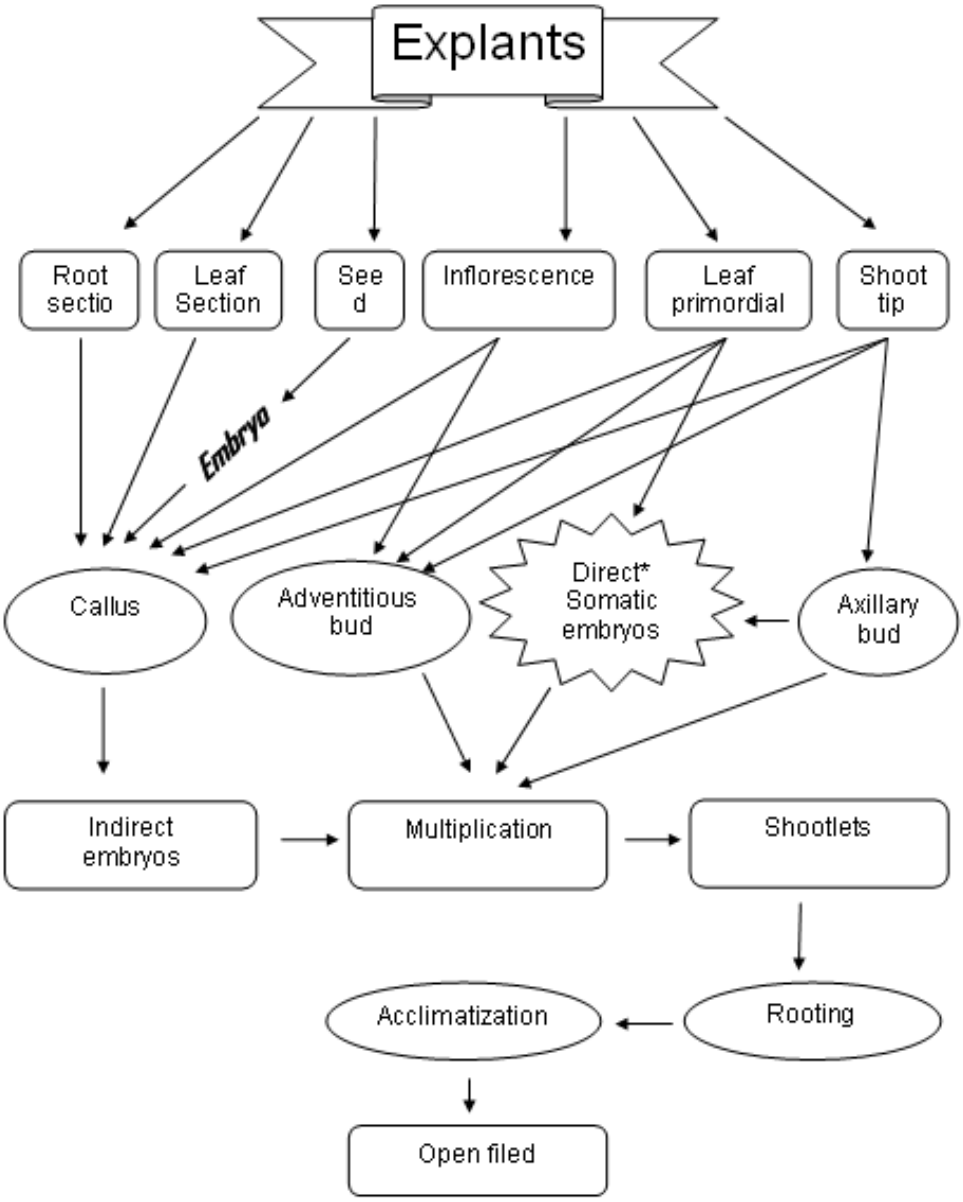

Fig. 1. Novel pathway in date palm micropropagation (*).

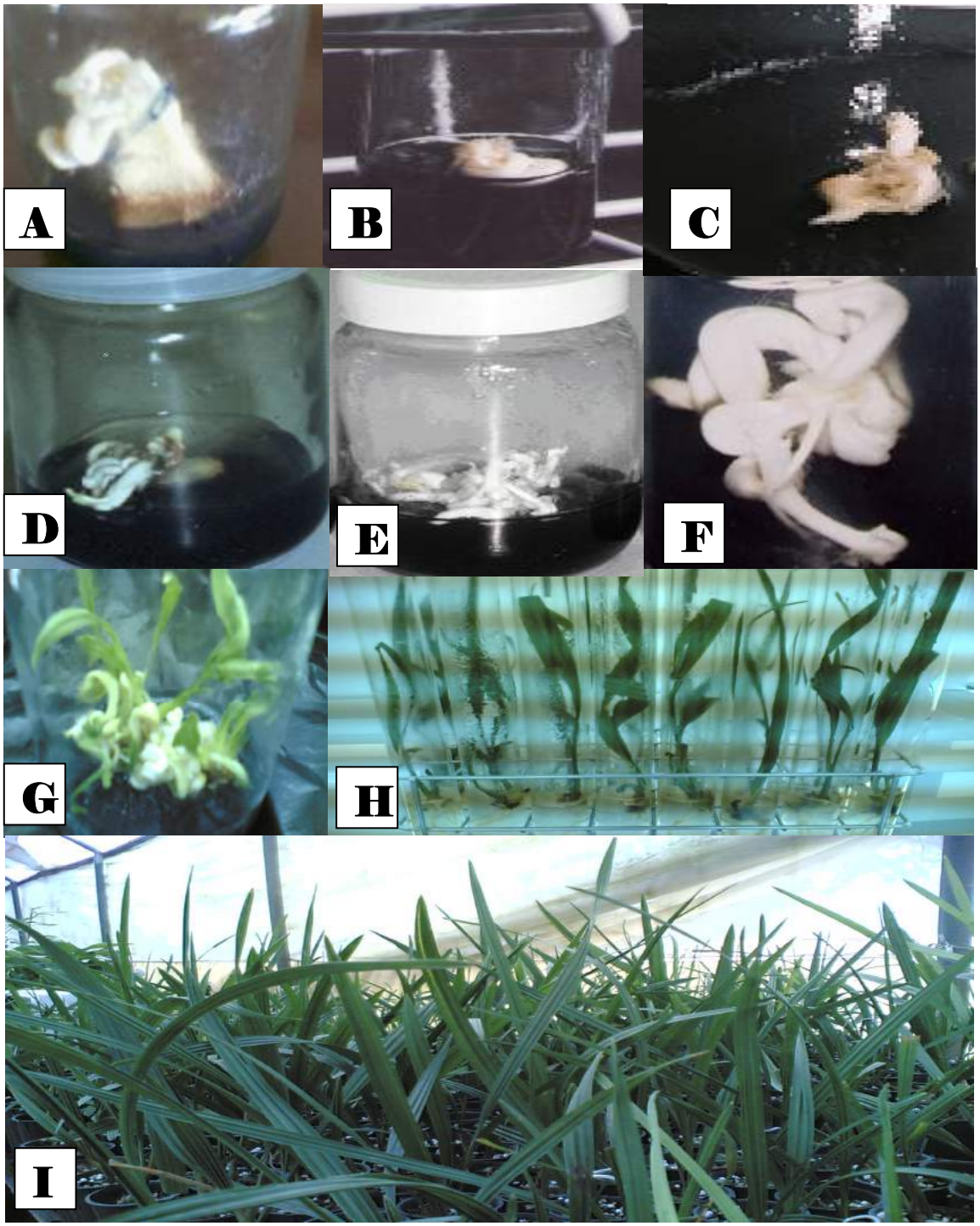

Fig. 2. Micropropagation of 'Medjool' via axillary bud proliferation derived embryos. A- Shoot tip after 3 recultures in dark conditions. B- Individually proliferated axillary bud under light conditions. C- Axillary bud changed in shape to initiate embryos. D- Axillary bud derived direct embryos. E- Embryos removal from the bud was grown. F- Repetitive embryos cycle. G- Embryos growth and development. H- Plantlets during rooting stage. I- Healthy plantlets after 3 months in acclimatization.

Exploring the Nigerian Date Palm (*Phoenix dactylifera* L.) Germplasm for In Vitro Callogenesis

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Keywords: *Phoenix dactylifera*, Genotypes, 2,4-Dichlorophenoxy acetic acid (2,4-D), callus formation, callus maintenance, morphotype

Abstract

Date palm is believed to have been introduced into Nigeria in the early 8th century by Arab traders from north Africa. Date fruits are a highly valued delicacy among many communities in Nigeria, especially during ceremonies and festivals. The national consumption of dates in 2009 is estimated at 8,958 metric tons which placed the country among the world top 10 consumers of date. Despite conducive soil and climatic conditions for date palm cultivation and the existance of local varieties with good fruit qualities, date palm cultivation is still at subsistance level and domestic production is estimated at only 1,958 metric tons. Attempts to improve the Nigerian date palm industry through the establishment of commercial date palm plantations have been hindered by lack of good planting materials. However, recent evaluation of the response of some of the Nigerian date palm cultivars to 2,4-D induced callogenesis demontrated the high propensity of the genotypes to in vitro culture, with more than 50% embryogenic callus formation on modified MS supplemented with 50 µm 2,4-D in all the genotypes tested. Further research is needed to complete and optimise this protocol in order to solve the problem of date palm planting materials in the country.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is believed to have been introduced into Nigeria in the early 8th century by Arab traders from north Africa, where it is traded in exchange for the dry leaves of the Henna plant (*Lawsonia inermins*), a plant widely used for body decoration by women in many parts of the world. Date fruits are a highly valued delicacy among many communities in Nigeria and enjoy a great spiritual and cultural significance. Nigerian date consumption as at 2009 is estimated at 8,958 t out of which 78% (7,000 t) were imported from Niger republic and North Africa via the trans-Sahara trade route. This value is significant when compared with 10,000 t imported annually by the United Kingdom which is among the 4 leading importers of date (FAO, 2003). However, only 22% (1.958 t) of the dates consumed is produced locally and this value is far below the annual production of Mexico (3,600 t) which is the least among 18 leading producers of dates (FAO, 2003).

In Nigeria, datepalm thrives well in the semi-arid region (12 to 14°N and 2 to 14°E). This area forms an undulating plain with general elevation ranging from 450 to 700 m and is covered by ferruginous tropical siols characterised by a sandy-fixed undulating topography and is characterised by wet (June to October) and dry (November to May) seasons (Mortimore, 1989). The prevailing soils and climatic conditions in this region support the growth of diverse date palm ecotypes and double fruiting per season with the second fruiting terminating at kalal stage due to high humidity (June-August). Date palm cultivation is still at the subsistance stage, where diverse cultivars are grown in homesteads and few ochards by local date growers. Previous attempts to improve date palm cultivation through the establisment of commercial plantations were not successfull

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due to the limited number of planting materials. Tissue culture remains the most efficient alternative for rapid mass propagation of selected date palm genotypes. Protocols for in vitro regeneration of date palm based on somatic embryogenesis or organogenesis were reported (Tisserat, 1979; Zaid and Tisserat, 1983; Showky and Mahmoud, 1998; Al-Khalifa, 2004; Eke et al., 2005; Asemota et al., 2007; Ahmad et al., 2009). However, genotype specificity in their response to in vitro regeneration made it necessary to develop a protocol for every date palm genotype (Hesselman, 1997). Since embryogenic culture allows for cyclic recovery of more plants, regeneration via somatic embryogenesis is an attractive alternative for rapid clonal propagation of date palm. In date palm, callogenesis is a prerequisite step to achieving in vitro propagation via somatic embryogenesis.

The objective of this study was to assess the callogenic competence in selected date palm genotypes in response to 2,4-D treament.

MATERIALS AND METHODS

Callus Induction

Two- to three-year-old offshoots of female plants of 7 date palm genotypes (Table 1) were obtained from the germplasm bank of the National Institute for Oil palm Research, Date palm substation Dutse, Jigawa State (11.7°N; 9.3°E) in August 2008. After removing the mature leaves, offshoots were trimmed down to the apical bud region. Soft tissue segments measuring about 20-40 mm were cleansed with a soap solution and treated with 20% w/v benlate (Benomyl methyl1-(butylcartamoyl)-2-benzimidazole carbamate) solution for 60 min. The segments were surface sterilized by dipping in 70% ethanol for 1 min followed by immersion in 20% commercial bleach (3.5% Sodium hyphoclorite) for 15 min and rinsed 3 times in sterile distilled water. The surface sterilized segments were further cut into small pieces (10-15 mm) and inoculated on modified MS supplemented with 2,4-D (50, 100, 150 or 200 μM), KH_2SO_4 (170 mg/L), and activated charcoal (250 mg/L) was added in the medium to check the browning of the explants. The pH of the medium was adjusted to 5.8 with 1 M NaOH and solidified by adding 0.8% agar-agar (BDH, England), before autoclaving at 121°C and 1.04 kg/cm^2 for 15 min. All cultures were raised in baby food jar (67×55 mm), each dispensed with 35 ml of the medium. Cultures were incubated at 29±1°C under continuous dark and were examined at an interval of 10 days. After 30 days of incubation, the explants were subcultured on fresh media containing the same concentations of 2,4-D and incubated for another 30 days. After 60 days of culture the number of segments forming callus were recorded and expressed as percentage callus induction.

Callus Maintenance

White compact callus was selected and subcultured on MS media supplemented with 50, 100, 150, 200 μM 2,4-D. For each treatment, callus with an average fresh weight of 500 mg was equally divided into 5 masses and incubated in a baby food jar containing 35 ml of the media. Each treatment had three replications laid in a completely randomised design. All treatments were cultured in the dark for 30 days. Increase in the fresh weight of the callus was recorded and the proliferation coeffecient was culculated. Data obtained were subjected to analysis of variance (ANOVA) (SAS, 1998) and means were seperated using Duncan's multiple range test.

RESULTS

Callus Induction

A preliminary experiment showed that fungal contamination in leaf segments obtained from offshoots was as high as 90% and can be reduced to <10% by treating the explants with 20% benlate for 60 min. Explant cultured in the dark on 2,4-D free medium did not produce callus. The majority of the explants turned brown and showed intense

oxidation and died after 4 weeks of culture. Explant cultures on the induction media containing 2,4-D enlarged in size and innitiated a white-brown colored callus at the cut edges after 2 months of culture in the dark (Fig. 1). All the datepalm genotypes tested in this study demonstrated optimum response to callus induction when cultured on induction medium containing 50 µM 2,4-D (Table 2). In general, the response of the genotypes to callogenesis increased when the concentration of 2,4-D was increased from 50 to 200 µM except in 'DPP4' and 'DPP5' for which the increase in the concentration of 2,4-D from 150 to 200 µM did not result in any increase in response to in vitro callogenesis. There were signicant differences among the genotypes in their response to callogenesis (Fig. 2). 'DPP1W' (74.88%) and 'DPP8' (74.22%) produced the highest percentage of callus induction and were different ($P<0.0001$) from 'DPP9' (69.91%). Differences among the four 2,4-D concentrations tested in this study were also significant (Fig. 4). Among the 2,4-D concentrations tested, 200 µM (85.25%) produced the highest percentage callus induction and the number of responding leaf segments significantly decreased with reduction in the concentration of 2,4-D.

Callus Proliferation

In order to asses the effect of 2,4-D on the proliferation of embryogenic callus in datepalm, white compact callus masses of average weight 100 mg were subcultured on media enriched with 2,4-D at the same concentrations used for callus induction for 30 days. On the basis of the increase in callus fresh weight the treatments were grouped into two; in the first group (50-100 µM 2,4-D) the callus fresh weight increased by 1.0- to 2.5-fold and in the second group (150-200 µM) the callus weight increased by 3.0 to 4.5 (Table 2). Significant differences were observed among the 2,4-D concentrations (Fig. 5) indicating that the increase in callus fresh weight was influenced by the concentration of 2,4-D. The highest proliferation was recorded with 200 µM and the proliferation correspondingly decreased when the 2,4-D concentration was reduced. Significant differences were also observed among the genotypes in their response to in vitro callus proliferation (Fig. 3) providing an insight that callus proliferation was in one way or the other influenced by the date palm genotype.

DISCUSSION

Mass propagation and genetic improvement of date palm requires the development of a reliable in vitro regeneration system. Since callogenesis is a prerequisite step to achieving in vitro propagation via somatic embryogenesis, development of protocol for efficient callogenesis is a critical stage in achieving rapid propagation of genotypes of interest. In this study, the seven date palm genotypes effectively produced embryogenic callus from immature leaf segments in media containing 2,4-D. In the case of date palm, leaf segments were reported to be the most competent tissue to form callus (Gueye et al., 2009). Callogenesis in date palm leaf segments in the presence of 2,4-D is characterized by two key events; the activation of fascicular paranchyma (FP) and reinitiation of the cell cycle by callogenic perivalcular sheath cells (PSCs) leading to dedifferentiation and callus formation (Gueye et al., 2009).

Due to its stability 2,4-D has been used in the induction of callogenesis in a wide range of species (Gaj, 2004; Ali et al., 2008). All the 2,4-D concentrations used in this study produced callogenesis >50%, demonstrating the efficacy of 2,4-D in the induction of callogenesis in date palm. This finding coincides with the report of Sane et al. (2006) and Othmani et al. (2009) on callogenesis in leaf segments of *P. dectylifera.* Auxin mediated callus induction has been linked to certain factors which may trigger the complete chain of events that influence the ability of cultured cells to grow in an organized fashion. The presence of specific receptors, that reside either on the cell membrane or within the cytoplasm (Mockeviciute and Anisimoviene, 1999). A specific binding site for both auxins has been identified (Kim et al., 2001). A class of proteins called expansins mediates the proton ability to cause cell wall loosening by breaking the hydrogen bonds between the polysaccharide components of the wall (Cosgrove, 2001).

Proton (H+) pumping and lowering of cytosolic pH result in an elevation of intracellular calcium level (Shishova et al., 1999). Both cytosolic pH and calcium ions have been associated with early auxin action (Zhang, 2003). Calcium ions, either themselves and or along with calcium binding proteins e.g., calmodulin activate the protein kinase cascade which in turn activates other proteins, including the transcription factors (Wagner, 2001). These factors presumably interact with the auxin-response elements and regulate the expression of auxin-inducible or auxin-responsive genes and exert its effect on the cell cycle and stimulate cell division (Johri and Mitra, 2001).

The current study has shown that callogenesis in date palm increases with a corresponding increase in the concentration of 2,4-D from 50-200 µM. This phenomenon was also reported to be more vigorous in date palm leaf segments cultured on media enriched with 50-200 µM 2,4-D (Othmani et al., 2009) and 54-270 µM NAA (Gueye et al., 2009).

Callogenesis was to a great extent influenced by the date palm genotypes. For example, callus induction and proliferation were >74% and >3.3-fold respectively in 'DPP1W' and 'DPP8', these values were significantly higher ($P>0.001$) than results obtained in the other five genotypes tested in this study. The type of callus tissue developed was also found to be genotype dependent. While white and compact callus was common to 'DPP1W', 'DPP2W', 'DPP4', 'DPP8' and 'DPP9', a mixture of white and brown compact callus was a common feature in 'DPP3' and 'DPP5'. Similar observations were reported in African cassava by Atehnkeng et al. (2006). Variation in the response of the date palm genotypes to in vitro callogenesis could probably be due to their physiological differences, particularly the endogenous IAA levels. Endogenous IAA levels were demontrated to be the main difference between leaf segments with various grades of callogenic competence in date palm (Gueye et al., 2009).

In this study all the date palm genotypes demonstrated a high success for callogenesis in leaf segments obtained from 2-3-year-old offshoots. These genotypes exhibited optimum callogenesis when culture on modified MS supplented with 50 µM 2,4-D and their response increases with an increase in the concentration of 2,4-D, demonstrating their high propensity to in vitro callogenesis. Further research is needed to complete and optimise this protocol in order to solve the problem of date palm planting materials in the country.

ACKNOWLEDGEMENTS

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Tables

Table 1. Fruit phenology and 2007 average yield/plant of the date palm genotypes used for the in vitro studies in 2008.

Genotype	Fruit type	Fruit colour	Seed	Yield/plant (kg)
DDP1W	semi dry	dark brown	small	79.8
DDP2W	semi dry	light brown	medium	106.3
DDP3	dry	dark red	meduim	50.8
DDP4	soft	light brown	small	86.1
DDP5	semi dry	light brown	small	63.2
DDP8	soft	dark brown	small	68.3
DDP9	dry	dark brown	small	94.0

Table 2. Response of datepalm genotypes to in vitro callogenesis from leaf segments obtained from offshoots in 2008 under different 2,4-D regimes.

Genotype	50 µM (2,4-D) % CI means±sd	100 µM (2,4-D) %CI means±sd	150 µM (2,4-D) %CI means±sd	200 µM (2,4-D) %CI means±sd	Mt
DPP1W	50±7.29	77±8.77	79±10.87	92±6.07	wc
DPP2W	62±9.64	69±9.47	78±11.52	85±9.47	wc
DPP3	54±10.50	75±12.66	69±12.18	84±7.81	wc,b
DPP4	57±9.64	66±11.12	85±11.05	85±9.40	wc
DPP5	59±12.12	66±6.01	79±7.11	79±7.24	wc,b
DPP8	59±11.20	75±9.79	72±7.95	91±5.93	wc
DPP9	55±9.22	69±4.81	76±4.97	98±4.82	wc
Genotype	PC means±sd	PC means±sd	PC means±sd	PC means±sd	-
DPP1W	2.67±0.30	3.06±0.35	3.09±0.48	4.36±0.48	wc
DPP2W	2.14±0.42	2.78±0.50	3.07±0.15	3.13±0.94	wc
DPP3	2.50±0.28	2.49±0.16	2.95±0.60	3.27±0.46	wc
DPP4	2.45±0.18	2.80±0.18	4.09±0.42	4.00±0.38	wc
DPP5	2.35±0.06	3.07±0.24	3.45±0.34	3.96±0.56	wc
DPP8	2.38±0.09	2.45±0.15	3.53±0.33	5.16±0.56	wc
DPP9	2.34±0.12	3.62±0.38	3.94±0.49	4.45±0.38	wc

Key: CI-callus induction, PC- Proliferation coefficient, sd- standard deviation, Mt-callus morphotype
Proliferation Coefficient = $\frac{\text{Fresh weight (30 days after subculture)}}{\text{Fresh weight (before subculture)}}$

Figures

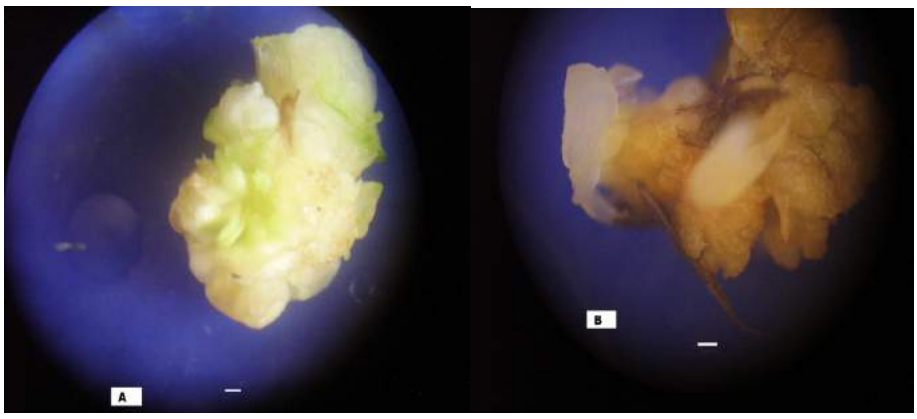

Fig. 1. Callus induction from (10-15 mm) long juvenile leaf segments of date palm (*P. dactylifera*). A- white compact embryogenic callus excised from leaf segment of 'DPP1W' after cultivation on modified MS supplement with 50 µM 2,4-D for 60 days. B- White and brown compact callus from juvenile leaf segment of 'DPP3' after cultivation on modified MS supplemented with 50 µM 2,4-D for 60 days.

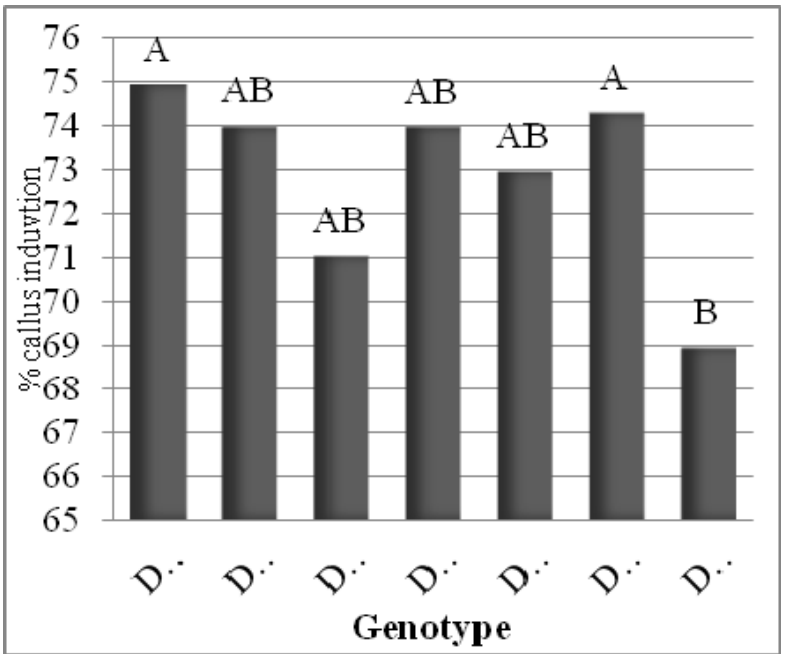

Fig. 2. Effect of genotype on the in vitro callogenesis in date palm.

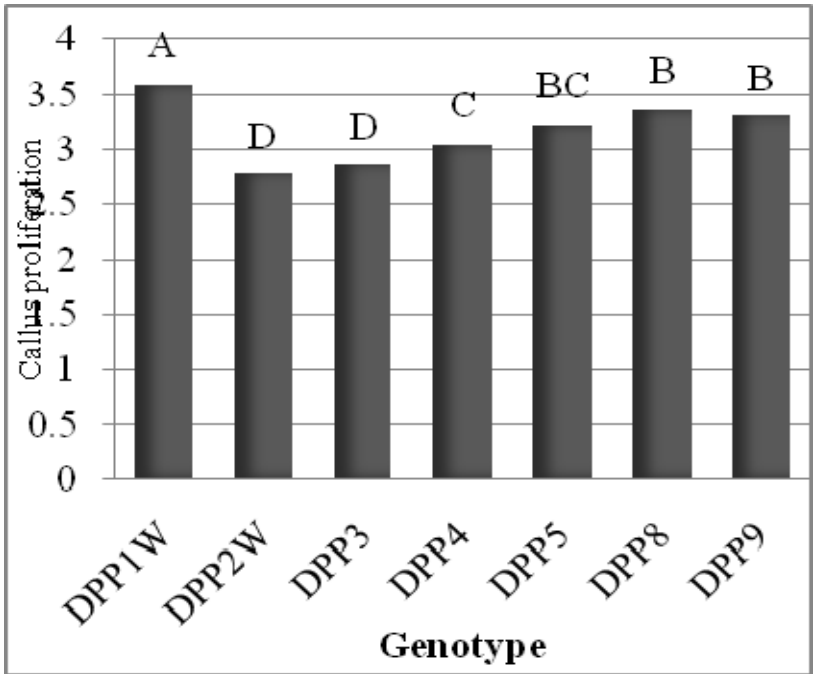

Fig. 3. Effect of genotype on callus proliferation 30 days after subculture.

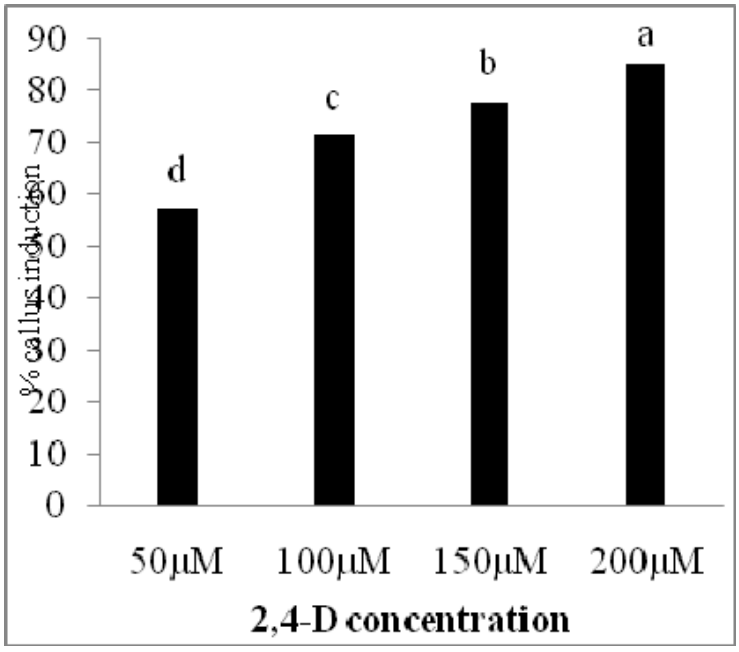

Fig. 4. Effect of different concentrations of 2,4-D on callogenesis in date palm.

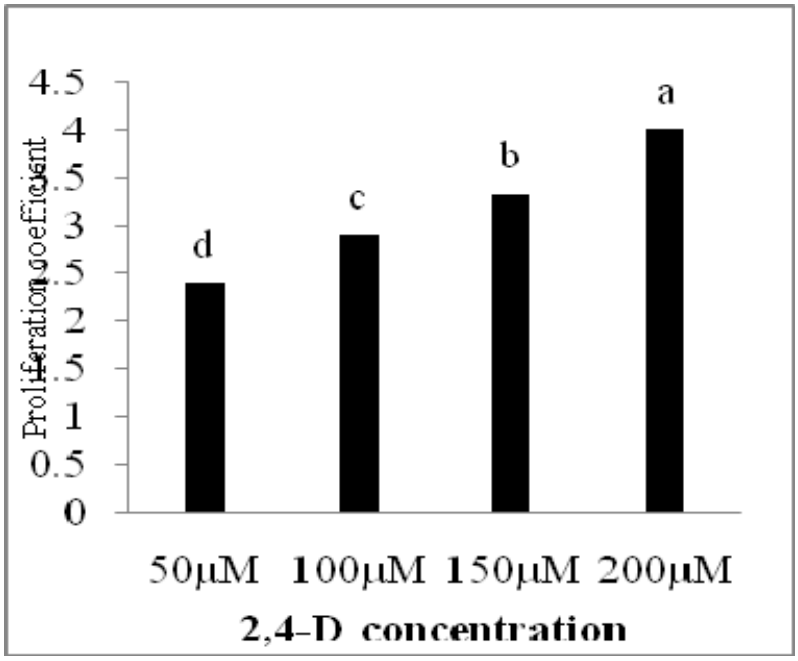

Fig. 5. Effect of different concentrations of 2,4-D on callus proliferation 30 days after subculture.

Importance of Protoplast Culture in the Genetic Improvement of Date Palm (*Phoenix dactylifera* L.)

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Keywords: Embryogenic callus, protoplast, cell division, microcalli.
Abbreviations: 2,4-D: 2,4-dichlorophenoxyacetic acid; IPA: Isopentenyl adenine acid; MS: Murashige and Skoog; PAS: Periodic Acid Schiff; NBB: Naphthol Blue Black

Abstract

Totipotent protoplasts are considered as a very important tool for plant genetic improvement. Protoplast were isolated from embryogenic calli in both 'Deglet nour' and 'Takerboucht' genotypes, calli were initiated from shoot apical tips of young offshoots of adult female trees growing in the field under natural conditions.

Embryogenic calli were obtained from shoot apical tips of offshoots excised in small pieces cultured on solid medium with low concentrations of growth regulators. The calli formed were friable white and yellow nodular. The isolation of protoplasts was achieved by transferring the plant material in enzymatic solutions in the dark. The yield of protoplasts obtained was sufficient to initiate a protoplast culture. Generally, the number of protoplasts obtained was more than 1.5×10^6. The protoplasts were cultured in both liquid and nurse culture. In terms of cell division rate, cell division was induced in both liquid culture and on nurse culture, but the best culture system was the feeder layer. The dividing cells developed to microcalli, which developed to calli on modified Murashige and Skoog medium. Calli were picked up and transferred on regeneration media to initiate plant organs.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), dioecious and diploid (2n=2x=36), is an important monocot cultivated in the south of Algeria, mainly in the southeast as Biskra and in the southwest regions as Adrar. This tree grows in arid lands in high temperatures that could be higher than 40°C especially in the summer and in soil with a high level of salts. It offers date fruits as staple food and quite funny ecological conditions for other cultivated crops as tomatoes, potatoes. In spite of it being an important economic plant in our country, this species is threatened by numerous diseases like Boufaroua, caused by *Oligonychus afrisiaticus*. It attacks especially the date fruits and can lead to fewer yields (Sedra, 2001); Bayoud, a fungal disease which causes vascular wilt of date palm trees by *Fusarium oxysporum* sp. *albedinis* living in the soil (Brac de la Perriere et al., 1995).

Therefore, it is very important to implement a breeding program for producing resistant plants with good quality of fruits. Generally, the classical cross breeding of this species can take more than twenty years to create a new variety. On the other hand, the number of the offshoots used for date palm distribution is less and insufficient (between 3 to 15 offshoots per tree) during its whole life per variety and per tree (Bouguedoura, 1991).

In our laboratory, we developed a protocol for plant production by somatic embryogenesis from vegetative and floral explants and from a resistant and a sensitive cultivar many plantlets were obtained. Recently we developed a protocol for protoplast

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isolation and culture to obtain young plantlets which could have a genetic character of resistant varieties and sensitive ones with good quality of fruits.

There are many reports on somatic hybridation used in other monocots as in banana (*Musa* spp.) (Assani et al., 2005), in rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) (Kisaka et al., 1998).

In the present paper, we studied the isolation of protoplasts from embryogenic calli and their behaviour in culture. We discussed the optimization of microcalli production onto two different culture systems; nurse culture and liquid medium and the capacity of this microcalli to induce calli with regeneration capacity to initiate plantlets on regeneration medium.

MATERIALS AND METHODS

The offshoots of two date palm varieties were tested: 'Deglet nour', a sensitive variety in the southeast as Biskra and a resistant one 'Takerboucht', from Adrar in the southwest.

'Deglet nour' is a cultivar which has the most soft date quality but is very sensitive to Bayoud disease and 'Takerboucht' produces less good fruit but is resistant. The offshoots were grown in natural fields (3 to 6 kg weight, 20 cm diameter, 120 to 150 cm tall) obtained from adult female trees. Shoot tips of both genotypes were used as donor material. To obtain callus culture, both growth regulators 2.4 D and IPA were required.

Preparation of Embryogenic Calli

Date palm callogenesis was achieved on solid Murashige and Skoog medium (1962), the offshoots were dissected by knives, the dry and lignified leaves were eliminated until the soft part (shoot tips). The shoot tips of offshoots were sterilized with 0.3% benlate (methyl [1-(butylcarbamoyl) benzoimidazol-2-yl] aminoformate) (DuPont, France) for 30 min, washed with an aqueous solution of 5.4% (v/v) sodium hypochlorite in water with two drops of Tween 20 for 45 min. After sterilisation, the tip was rinsed 3 times of 10 min each with distilled water. After sterilisation, the dissection of offshoots was pursued until the soft, white part (shoot apical tips about 5 cm long, 3 cm diameter). The shoot apical tips were excised in small pieces (5-10 mm long) and cultured in test tubes (20 ml, 1 piece) and on petri dishes (9.5 cm diameter) on solid medium M_a (20 ml, 10 pieces per petri dish). The medium consisted of MS salts (Murashige and Skoog, 1962) supplemented with Morel vitamins (Morel and Wetmore, 1951), 87 mM sucrose, 9.0 µM 2.4-D, 14.76 µM IPA and 7 g L^{-1} agar (Sigma, USA). The pH was adjusted to 5.7 before autoclaving (20 min, 120°C, 1 bar). The cultures were kept at 27°C in the dark. The explants were transplanted every 4 weeks on the same medium.

Calli Histological Analysis

The histological analysis concerned embryogenic calli and microcalli. They were fixed in a solution consisting of 100 ml, 4 ml of a 25% glutaraldehyde solution, 50 ml of phosphate buffer pH 7.2, 20 ml of 10% paraformaldehyde solution, 1 g of caffeine and 26 ml of distilled water (Schwendiman et al., 1988). A vacuum passage of calluses was released.

Dehydration was made with ethanol progressive degrees followed by impregnation in methyl methacrylate, embedded in epoxy resin (Historesin from Reichert-Jung). Sections, 3 µm thick, were obtained and then stained with Periodic Acid Schiff (PAS) and Naphthol Blue Black (NBB) at 60°C and schiff product in the dark as described by Fisher (1968).

Isolation and Purification of Protoplasts

Selected calli with embryogenic parts prepared from shoot tips of offshoots were used as donor material for the isolation of protoplasts.

About 1 g f.wt. of embryogenic calli was placed in 15 ml of an enzyme solution with 1.5% (w/v) cellulase RS (Yakult Pharmaceutical Ind., Japan), 0.15% (w/v) (Kyowa

Chemical Products Co, Osaka Japan), 0.2% (w/v) hemicellulase (Sigma, USA), 204 mM KCl, 67 mM $CaCl_2$ (pH 5.6). The enzyme-callus mixture was incubated without moving in darkness overnight (12-15h). This mixture was filtered through a 100/25 µm metallic mesh combination to remove cell colonies of calluses. Protoplasts were collected in a solution composed of 204 mM KCl, 67 mM $CaCl_2$ by centrifugation at 66 g for 5 min. The pellet was suspended twice in the same washing solution composition by centrifugation (66 g, 5 min). The yield of protoplasts obtained in each isolation was estimated with a Nageotte hematocymeter. The cell wall regeneration was detected by calcofluor brightener stain (Nagata and Takebe, 1970).

Culture of Protoplasts

Fresh protoplasts isolated were cultured at a density of $\geq 10^6$. Two types of culture systems were tested; liquid medium and nurse culture on filter nitrocellulose paper.

The liquid medium (Mb) consisted of MS salts, vitamins of Morel, 0.68 mM glutamine, 117 mM sucrose, 0.4 mM glucose, 0.5 mM MES, 1.9 mM KH_2PO_4, 9.0 µM 2,4-D, 14.76 µM IPA and 250 mg L^{-1} polyethylene glycol 4000 (PEG). The pH was adjusted with NaOH (0.1 N) and sterilised by filter (0.22 µm millex GS filters, Millipore Corporation).

About 3 ml of protoplast suspension in liquid medium was taken and transferred to the petri dishes (5.5 cm diameter) and correctly sealed with clingfilm and maintained at 27°C in the dark. The nurse culture was prepared by using chopped embryogenic callus of 'Deglet nour' with high embryogenic capacity as feeder layer. The PCM liquid medium (double strength) was prepared the same day of the protoplast isolation. The PCM (Mc) composition was, MS salts, vitamins of Morel, 2.8 mM glucose, 278 mM maltose, 170 mM sucrose, 9.0 µM of 2,4-D, and 2.5 mM Myo inositol (pH 5.7), it was sterilized by filter (0.22 µm millex GS filters, Millipore Corporation). Suspension calli were obtained by addition of 100 ml of PCM medium to obtain 0.2%/Agarose mixture. 1.2 g of Agarose sea plaque (Sigma, USA) was dissolved in 100 ml of distilled water, the pH was adjusted to 5.7 and autoclaved at 120°C during 20 min. Aseptically, when the temperature of agarose decreased to 30-40°C, the liquid medium was slowly mixed with 100 ml PCM containing nurse callus cells. The mixture was quickly distributed into small petri dishes (5.5 cm diameter). After an average of one hour, the medium was covered with sterilised nitro-cellulose filter (AA, type Millipore corporation). All cultures were maintained at 27°C in the dark. The behavior of protoplasts in culture was studied under microscopic observation and cell wall regeneration was determined by calcofluor white brightener stain (Nagata and Takabe, 1970).

The microcalli formed were cultivated onto callus induction medium containing MS salts with 13.5 µM 2,4-D and 14.76 µM IPA; Morel vitamins and 3 g gelrite. The calli were transferred to the regeneration medium, it consisted of MS salts with 14.76 µM IPA and 1.4 µM 2,4-D. The cultures were transferred onto the medium without growth regulators and were kept in the dark at 27°C.

Data Collection and Statistics

Three independent assays were realised and the results obtained at each investigation were reported. We tested 10 petri dishes. The yield of protoplasts obtained was calculated after 3 independent countings of viable protoplasts. The rate of dividing cells was calculated after 1 week of protoplast plating. The number of microcalli induced were reported after each subculturing.

RESULTS

This investigation presents a protocol followed to obtain a high intensity of embryogenic calli and how to isolate protoplasts from them and on the other hand, the most important factors influencing their behaviour in culture for inducing microcalli and their capacity to develop plant organs.

Explants Callogenesis

Explants of the 'Deglet nour' genotype showed more callogenesis capacity than 'Takerboucht' explants on medium containing 9.0 µM 2,4-D and 14.76 µM IPA, we noted that many other concentrations of growth regulators were tested (data not shown), callogenesis depended on the season of culturing. When offshoots were taken from palm groves in spring, the explants obtained from shoot tips showed more fast activity in callogenesis than in the other seasons. After 3-6 months of culture, yellow nodular/compact and white friable calli were obtained in both genotypes, 'Deglet nour' and 'Takerboucht' (Fig. 1a).

Concerning the rate of calli obtained, it was about 50% of calli in 'Deglet nour' and to 36% of calli in the 'Takerboucht' genotype.

Histological sections of this callus showed the formation of many individual spherical structures with different diameter. These cell aggregates were composed of small meristematic cells with rich and dense cytoplasm, a big nucleus containing 1 or 2 nucleolus. The number of these spherical structures was raised; they were most likely to form embryogenic calli (Fig. 1b).

Protoplast Isolation and Behavior in Culture

To induce microcalli formation from protoplasts and testing their capacity to develop in plant organs, the following factors were assessed; nature of calli, nurse cells, culture system.

Under the present experimental conditions, the yield of protoplasts obtained was sufficient to initiate a protoplast culture. Generally, the number of protoplasts with circular shape obtained was more than 1.5×10^6 (Fig. 1c) in both culivars. Neverthess, the protoplasts of 'Takerboucht' had a brown color and 'Deglet nour' protoplasts were transparent.

After culture in two liquid media and on filter nitrocellulose, protoplasts of both varieties showed cellular wall induction with cell shape modification with time. Despite, the induction of protoplast division in both genotypes tested. The liquid medium showed a very low rate of dividing cells to undivided cells in comparison to those plated on a nitrocellulose filter.

Concerning the protoplast cultured on nitrocellulose filter paper, they showed many dividing cells, at the beginning, after 2 to 3 days of culture, protoplasts showed a shape modification from round to oval. With time, the cells revealed a second, third and several divisions in order to constitute a microcallus (Fig. 1d, e).

The number of microcalli obtained varied with the substratum nature (Fig. 2), it was higher on a nitrocellulose filter than in liquid medium, the average number was 16000 per petri dish on nitrocellulose and 300 in liquid medium after 3 months of culture, independent of the variety, 'Deglet nour' or 'Takerboucht'.

Microcalli Transformation

After subculturing, on the callus induction medium, the microcalli were transformed to very soft and friable white calli after 2 months on MS salts with 13.5 µM 2,4-D and 14.76 µM IPA; with nodular proembryogenic structures less than 2 mm of diameter. About 25% of the microcalli developed to callus.

These calli developed to somatic embryos onto medium containing MS salts with 14.76 µM IPA and 1.4 µM 2,4-D (Fig. 1f).

Placed onto medium without growth regulators, many somatic embryos showed mainly radicular parts at first in comparison to a few of them which expressed a caulinar part at first. We noted that somatic embryos which started with a radicular part did not develop any more caulinar parts.

DISCUSSION

In the present study, we developed a method for regeneration of protoplasts till expression of somatic embryos.

The first step is callogenesis induction; we noted that the rate of calli obtained in explants cultivated in spring was more important than others cultivated in autumn, winter and summer (Chabane, 1995). The calli activity was expressed on MS culture medium containing low concentrations of 2,4-D and IPA. This work confirms that the association of 2,4-D/IPA was more important for inducing a most important mass of calluses in date palm. Othmani et al. (2009) reported that 2,4-D was necessary to induce callogenesis and embryogenesis in date palm trees and the concentration required varied with Tunisian genotypes as 'Amsekchi' (Sané et al., 2006), 'Jihel', 'Bousthami noir' (Zouine and El Hadrami, 2007) and 'Deglet nour' (Fki et al., 2003).

This investigation presents a protocol followed to isolate protoplasts and the most important factors influencing their behaviour in culture and their capacity of regeneration of date palm trees. Our previous study showed that the mixture of cellulase RS, pectolyase and hemicellulase was more beneficial for the isolation of protoplasts from young leaves of offshoots and from calli in date palm (Chabane et al., 2007).

Concerning the effect of growth regulators on microcalli induction, it was observed that many microcalli were initiated on 2,4-D alone (9.0 µM of 2,4-D), in contradiction to Rizkalla (2007) who noted that microcalli initiation required a high concentration of 2,4-D as 100 mg and 3 mg of 2IP.

The critical step in this work was the induction of mitotic activity and their transformation to microcalli, which could be recalcitrant. A comparison from two culture systems used for regenerating protoplasts showed that the nitrocellulose filter allowed more mitotic activity and microcalli formation than liquid medium. The important division activity of protoplasts occurred with the feeder layer as embryogenic calli, which has efficient effects on mitotic activity on protoplast, then on microcalli production. It is important to note that the embryogenic calli could be a source of young meristematic cells, which could be a powerful tool for microcalli initiation. Many researchers reported this positive impact of embryogenic cells as nurse cells ten years ago. Since 1989, Eigel and Koop and Karp and Lazzeri (1992) published that nurse cells could produce growth promoting factors (GPFs) which affected protoplast divisions.

The use of nurse culture was reported as a tool to solve problems of regeneration of recalcitrant protoplasts (Horita et al., 2002; Sun et al., 2004). Recently, Assani et al. (2006) observed that the use of nurse culture significantly improved the protoplast dividing and microcalli formation in *Musa* spp.

Varotto et al. (2006) noted that in *Cichorium intybus*, microcalli developed into minicalli which produced buds via organogenesis, young differentiated buds from calli were transferred after 5 months of culture for rooting.

In our experimental conditions, microcalli obtained from dividing protoplasts developed very soft white calli and white somatic embryos.

Concerning the regeneration of somatic embryos, they initiated a radicular part. Nevertheless, a very low number of somatic embryos (2%) started with a caulinar part initiation and rooting for regenerating plantlets. In contrast, Yu et al. (2000) have also reported that somatic embryos developed from protoplast-derived microcalli or proembryos, but 33.1% of white somatic embryos regenerated into plantlets.

Then, it is very important to improve the calli regeneration process from protoplasts and their development into plantlets.

CONCLUSION

This study presented a method for isolation of protoplasts from embryogenic calli in date palm trees and all conditions used to induce their mitotic activity of cells and their transformation to microcalli culture media (1) enzymatic mixture, time of incubation solution, (2) the substratum of culture used for microcalli initiation and proliferation, (3) calli multiplication and regeneration to somatic embryos with large number with radicular part without culinary part except a few of them.

In the next study, it is important to improve the experimental protocol for protoplasts regeneration.

In order to find the most conditions which have a positive effect on the quality of microcalli to raise them and to induce calli with regeneration of strong somatic embryos, which could lean on caulinary production to radicular expression, it is important to focus on the growth regulators concentrations used for many shoot expression to raise the number of them and search the efficient method for optimization and conservation of protoplasts.

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Figures

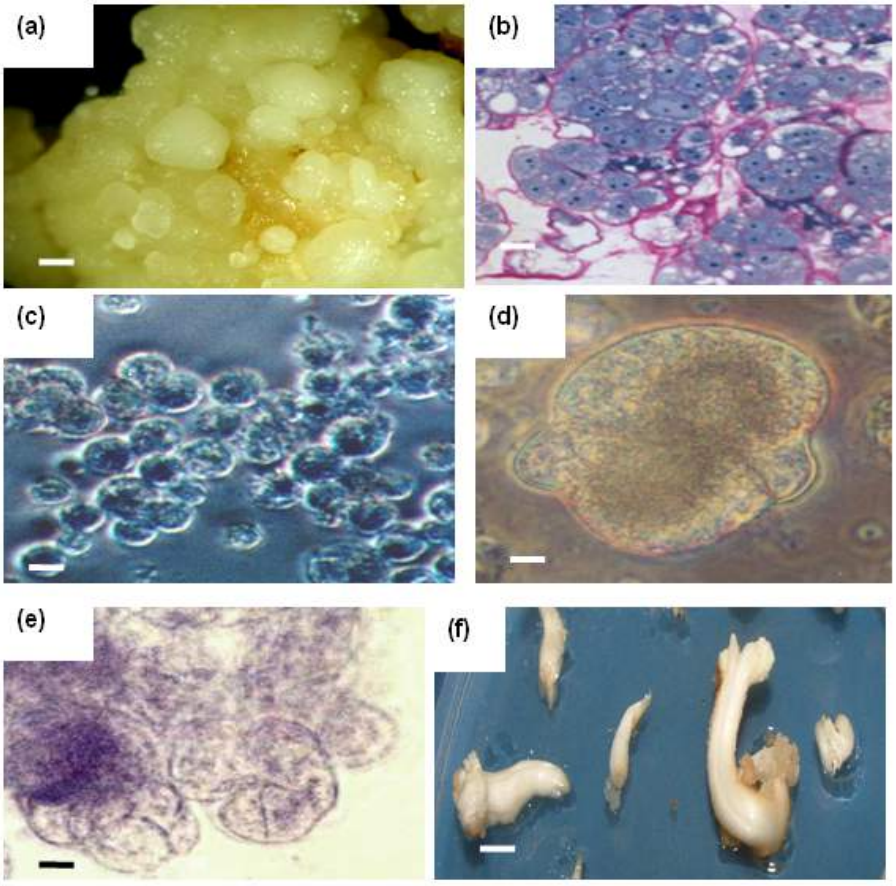

Fig. 1. (a) Embryogenic calli from shoot tip of 'Deglet nour' after 5 months of culture; bar 6 mm. (b) Histological structure of nodular calli showing cells with large nucleus with 1 to 2 nucleolus, very rich in proteins; bar 25 µm. (c) Protoplasts isolated from embryogenic calli of 'Deglet nour'; bar 30 µm. (d) Dividing cell one week after isolation; bar 10µm. (e) Microcalli formation; bar 0.25 mm. (f) Numerous somatic embryos regenerated from microcalli; bar 2 mm.

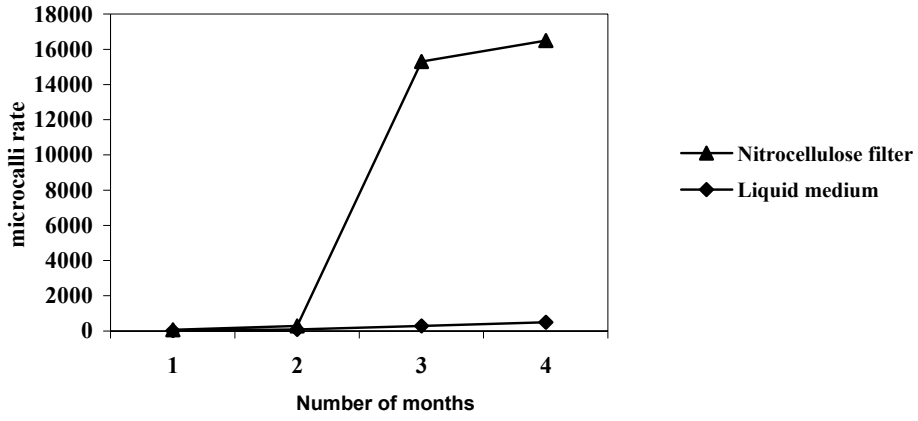

Fig. 2. The effect of the nature of the culture system on microcalli obtention. The rate of microcalli grows slowly in the 4th month on the nitrocellular filter and in liquid medium.

Cultivar Differences of Date Palm (*Phoenix dactylifera* L.) in Somatic Embryogenesis Micropropagation

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Keywords: date palm, micropropagation, somatic embryogenesis

Abstract

Date palm (*Phoenix dactylifera* L.) propagation commonly occurs via somatic embryogenesis. A similar nutrient medium composition is used for all date palm cultivars. The objective of this study is to evaluate the performance of two cultivars using the standard somatic embryogenesis nutrient medium composition. Callus was initiated from an in vivo actively growing cultivar ('Mishrig wad Khateeb') and a slowly growing one ('Mishrig wad Laggai') following the same steps. Callus growth prior to differentiation, expressed in fresh weight, frequency of initiation, number of resultant somatic embryos and time needed for their establishment were determined. Results indicated callus initiated two weeks earlier in MWK compared to MWL with significantly heavier weights. Similarly, significant differences exist in the overall number of somatic embryos formed. There were no conspicuous differences in rooting of the two cultivar plantlets. There were cytological and chemical differences between the two cultivars.

INTRODUCTION

Four decades have passed since the first attempts of the date palm tissue culture. Presently, the application of tissue culture techniques for date palm are an economically reliable technique that enables large scale multiplication of genetically uniform healthy selected cultivars throughout the year (Zaid and de Wet, 2002). The most commonly used procedures for date palm micropropagation are organogenesis and somatic embryogenesis.

More than 5000 cultivars exist in different date palm growing countries in the world. The cultivars vary in their morphological features and production characteristics (Zaid and de Wet, 2002). The morphological and physiological qualities are due to interaction effects to environmental conditions influencing the biological nature of the tree (Elshibli, 2009). Despite this variability, the commercial date palm cultivars receive analogous management practices with variable response in different cultivars (Osman, 1984; Dawood and Ahmed, 2006).

In somatic embryogenesis, shoot tip explants are cultured on modified Murashige and Skoog (MS) medium containing 3 g L^{-1} activated charcoal; with 100 mg L^{-1}, 2,4-D and 3 mg L^{-1} N6-(2-isopentyl) adenine (2-iP). A prolific callus is produced after repeated reculturing for several months depending on the cultivar (Tisserat, 1979).

The variable response of date palm cultivar to somatic embryogenesis induction medium remains elusive. This investigation, therefore, was aimed to explore the basis of such variation between the fast growing (MWK) and slow growing (MWL) date palm cultivars.

MATERIALS AND METHODS

Shoot terminals were obtained from two- to four-year-old offshoots removed from 'Mishrig wad Khateeb' and 'Mishrig wad Laggi' date palm trees at Elgeraif Gharb South East of Khartoum. Offshoot leaves were removed acropetally, revealing the soot

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terminals. Shoot terminals of 10 mm or greater in length were excised and temporarily left in an in aqueous solution of 150 mg L^{-1} citric acid and 100 mg L^{-1} ascorbic acid until surface sterilization was carried out. All explants were surface sterilized by soaking in a 10% (v/v) sodium hypochlorite solution (containing one drop of Tween-20 emulsifier per 100 ml of solution) for 20 min and rinsed three times with sterile distilled water. The explants were trimmed out and fragmented to 1-3 mm^3 segments. One segment of shoot tip was placed over the surface of a solid nutrient medium in 25×150 mm culture tube. 100 cultures were initiated for each cultivar.

The medium was composed of Murashige and Skoog (1962) inorganic salts and in mg L^{-1}: 100, myo-inositol; 0.5, nicotinic acid; 0.5, pyridoxine; 1.0, thiamine; 2.0, glycine; 30000, sucrose and 7000, agar. The pH was adjusted to 5.7±0.2 with 0.1 N NaOH or 1 N HCl prior to the addition of agar.

The nutrient medium was dispensed into 25×150 mm culture tubes at a rate of 25 ml, covered with polypropylene Ka-puts closures and sterilized by autoclaving for 15 min at 121ºC and 1.05 kg cm^{-2}. Data recording, reculturing and subculturing was carried out every two months.

All cultures were incubated in complete darkness at 27±2°C till formation of nodular callus and then transferred to low light intensity of 1000 Lux illumination.

Small masses of friable embryogenic callus (2-4 mm in size) were transferred from the high auxin media to media containing 0.1 mg L^{-1} NAA for 2-3 months and the germinating somatic embryos were separated individually in culture vessels to reach a suitable size for acclimatization.

Tissues used in histological observation were fixed in FAA (formalin:glacial acetic acid:50% ethanol 5:5:90 (v/v/v)), dehydrated in ethanol series and embedded in paraffin. Embedded tissues were sectioned, 10-12 µm thick and stained with safranin and fast blue (Johansen, 1968). The cell sizes were determined with an ocular micrometer and photographed.

Chemical analysis of fresh date palm leaflets of the two cultivars for benzoic acid, 2,4-D were prepared for extraction for GLC analysis. Atomic absorption spectrophotometery and flame photometery were used to detect the levels of Na, K, Ca, Mg and P in leaf tissues AOAC (2000).

Data were collected and analyzed as a completely randomized design with cultivars as treatments. The analysis of variance was conducted by SPSS 12.0.1 for Windows to test the significance of variation between cultivars for each character within the period of data collection. The differences between individual cultivars were determined using the least significant difference (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Shoot tip explants of both cultivars cultured in medium containing a high level of auxin responded to callus induction. However, cultures of MWK responded to callus formation two weeks earlier than MWL. After few subcultures on the same medium shoot tip explants gave rise to yellowish callus (Table 1). The callus of MWK was actively growing (Fig. 3) and characterized by a heavier weight during the first 12 weeks compared to that of MWL. The callus weight of MWL surpassed that of MWK after 18 weeks of culture (Fig. 1). However, there was no significant difference between the callus weights of the two cultivars throughout the period of the study (Fig. 2). This was probably due to the discrepancy in the initial cultured tissues during subculturing and reculturing.

After repeated subculturing on high auxin medium, embryogenic nodular callus was initiated in 4 months in MWK and 8 months. The nodular callus was embryogenic in nature, friable and effortlessly propagated, elongated and germinated in both cultivars (Fig. 4). The nodular aggregates were multiplied and further differentiated in fresh medium. The percentage of cultures differentiated into embryos in MWK was significantly greater than that of MWL. Similarly, the number of embryos formed was also higher (Table 2). There was no difference in rooting percentage of the differentiated embryos. The roots of MWK, however, were extensive and stronger (Fig. 5).

In surveillance of rationale to cultivar differences, microscopic examinations of callus sections were prepared after four and eight weeks. The sections revealed differences between MWK and MWL date palm cultivars (Fig. 5). After four weeks the MWK callus tissues were loose and friable with densely cytoplasmic meristematic cells surrounded by highly vacuolated cells. The analogous cells in MWL were compact, small and surrounded by semi-vacuolated cells. These cells would probably proliferate into proembryos that give rise to bipolar structures which differentiate into plantlets (Tisserat and DeMason, 1980).

The differential response of MWK and MWL to callogenesis was presumably owed to genetic, compositional and morphological diversity of cultivars (Elshibli, 2009). Therefore, in search for an explanation, mineral contents of date palm cultivars were analyzed and shown in Table 3. The amounts of potassium, calcium, magnesium and phosphorus were similar in both cultivars. However, the sodium content in MWL was double that of MWK. This might affect the electrochemical potential across the cell membrane due to unequal charge distribution (Ting, 1985). Perhaps this would disrupt the equilibrium across the membrane resulting in the differential response.

Benzoic acid competes with the auxins by binding to plasma membrane competitively with the auxin. Thus, the presence of high levels of benzoic acid indicates inhibition of auxin activity. The presence of high levels of endogenous auxins, on the other hand, may enhance their action. According to these assumptions, analysis of benzoic acid and 2,4-D were conducted using GLC methods of analysis. However, these attempts failed, probably to the low content of these substances in these particular tissues that might need further concentration of extracts and/or the inefficiency of the method of analysis for the substances. Therefore, exploring these traits can be considered for further studies related to varietal differences.

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Tables

Table 1. Callus weights of date palm cultivars.

Time	Cultivar	
	Mishrig Wad Laggai	Mishrig Wad Khateeb
Initial	0.31±0.19	0.29±0.20
After 6 weeks	0.67±0.39	0.79±0.49
After 12 weeks	1.26±0.68	1.43±0.55
After 18 weeks	1.55±0.78	1.46±0.75
C.V.%	56063%	
$LSD_{0.05}$	0.2796	
SE±	0.1003	

Table 2. Rate of embryogenesis and number of embryos in 'Mishrig Wad Khateeb' and 'Mishrig Wad Laggai' date palm cultivars.

Cultivar	Embryogenic cultures %	Number of embryos
Mishrig Wad Khateeb	85.2 (66.3-95.8)*	162±25**
Mishrig Wad Laggai	44.4 (25.4-64.7)	87±15

* Confidence limits at 95%.
** Standard error.

Table 3. The mineral content of date palm cultivars.

Cultivar	Na%	K%	Ca%	Mg%	P%
Mishrig Wad Laggai	0.17	2.9	0.35	0.18	0.83
Mishrig Wad Khateeb	0.09	2.7	0.30	0.18	0.67

Figures

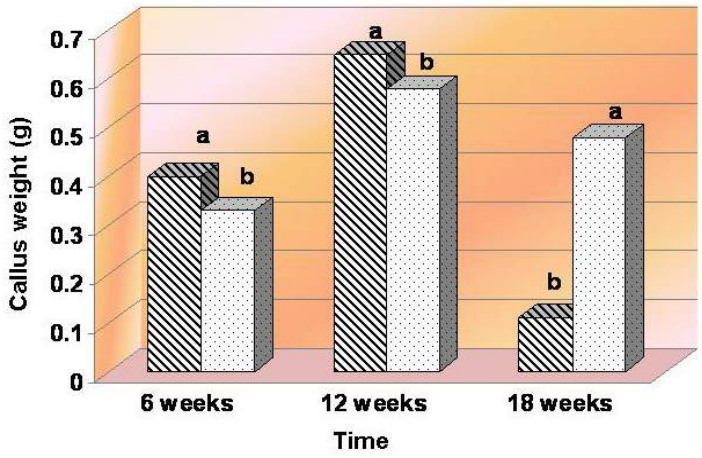

Fig. 1. Increase in callus weight of 'Mishrig wad Khateeb' ▨ and 'Mishrig wad Laggai' ▩ date palm cultivars. Same letter in column within a period were not significantly different at P=0.5.

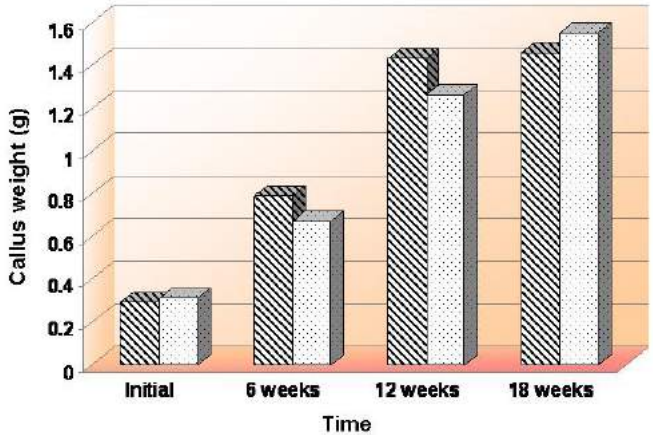

Fig. 2. Callus growth of 'Mishrig wad Khateeb' ▨ and 'Mishrig wad Laggai' ▩ date palm cultivars.

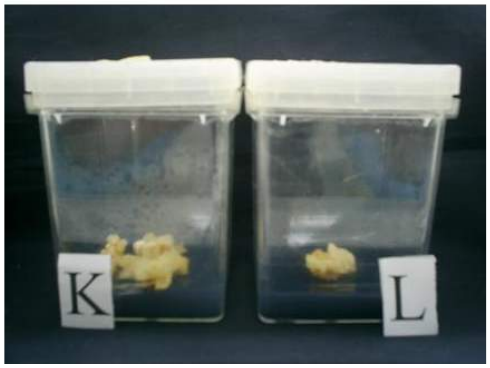

Fig. 3. 'Mishrig wad Khateeb' (K) and 'Mishrig wad Laggai' (L) callus growth. Data taken after 6 weeks in culture.

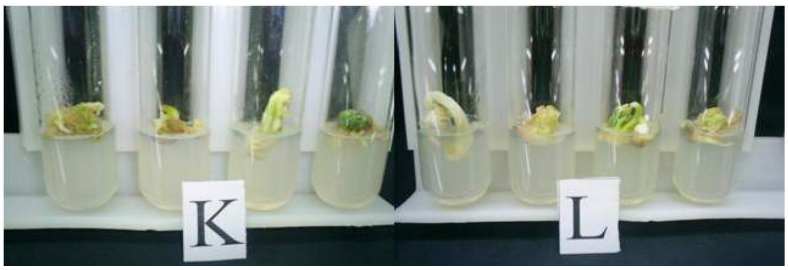

Fig. 4. Comparison of embryogenic response of MWK (K) and MWL (L) date palm cultivars.

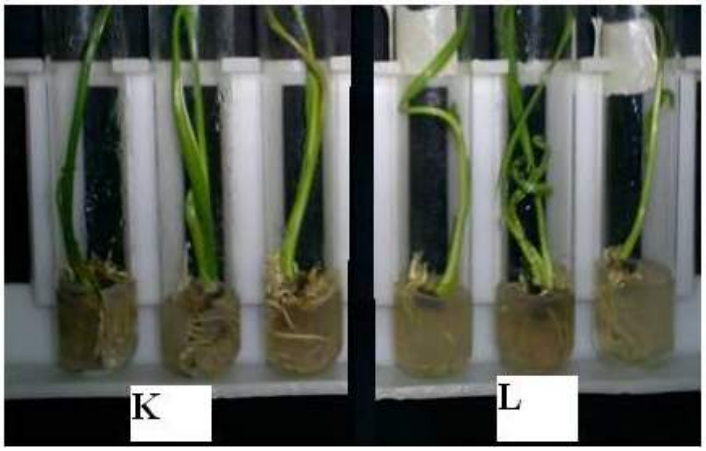

Fig. 5. Rooting of 'Mishrig wad Khateeb' (K) and 'Mishrig wad Laggai' (L) date palm cultivars.

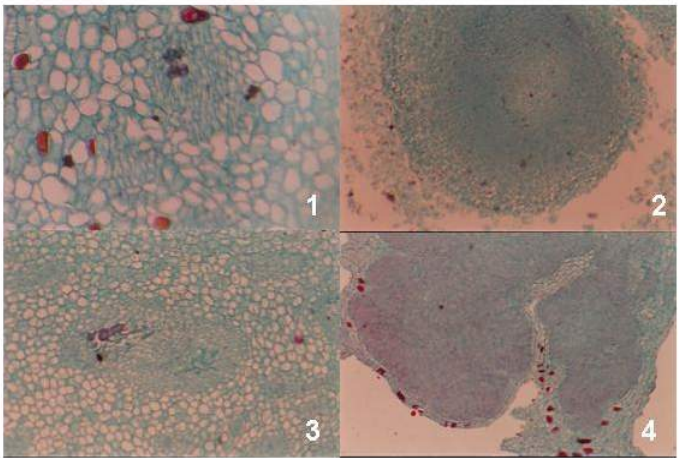

Fig. 6. Transverse section through embryogenic callus of MWK (1, 2) and MWL (3, 4) 4 weeks (1, 3) 25× and 8 weeks (2, 4) 10× in 100 mg L^{-1} 2,4-D medium

Histological and Biochemical Studies of Zygotic Embryogenesis towards improvement of Somatic Embryogenesis in Date Palm (*Phoenix dactylifera* L.)

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Keywords: date palm, zygotic embryogenesis, proteins of reserve, beams conducting

Abstract

In Algeria, the culture of date palm is subjected to many threats of which most worrying are the ageing of date palm orchards and Bayoud, vascular mortal disease of the date palm, caused by *Fusarium oxysporum* F. sp. *albedenis*. The methods of in vitro culture are currently adapted for the improvement of the culture and the fight against these threats.

Various techniques of micropropagation were largely developed on the date palm, of which somatic embryogenesis remains the most current. However, in the development of the somatic embryos, although relatively synchronous, a random remainder stays, in particular during the phase of germination. The objective of this paper falls under the comprehension of the embryonic zygotic development of the date palm, which has been studied very little, in order to better apprehend the problems relating to the process of somatic embryogenesis. Thus, our work was directed towards the deepening of knowledge of the stages of the formation and the maturation of zygotic embryogenesis towards the histological and biochemical plan.

During the development of the ovule fertilized in young seed, the embryonic bag undergoes several transformations. The cell egg or zygote which appears at the 12th day after pollination suddenly shows the first transverse division leading to two superimposed and unequal cells. Successive divisions allow the formation of a globular embryo of form two months after fecundation. The development of the embryo, relatively slow, thus passes by a stage heart form to bilateral symmetry; two side territories being used for the installation of the cotyledon entirely sheathing. Continuous divisions of the cells of the embryo lead to an increase in size and embryonic complexity, in order to set up the embryonic axis and the cotyledonary limb. The embryonic axis then consists of a shoot meristem surrounded by the cotyledon and the first leaf post cotyledonary initiated during the embryogenesis which is traversed by procambial beams. The distinction between the hypocotyl axis and the very reduced root meristem is difficult.

The electrophoretic study of mature proteins of zygotic embryos of two varieties, 'Deglet nour' and 'Ghars', highlights several bands whose majority have their counterparts in the profiles of the zygotic embryos of *Washingtonia filifera* Will and *Elaeis guinneensis* (Chandra and DeMason, 1988; Morcillo, 1998). These results suggest similarities in the composition out of proteins of reserve at these various species of palm trees.

The germination and the viability of the somatic embryos thus depend on the installation of the proteinic reserves and especially of the conducting beams which connect the shoot part and the root part.

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INTRODUCTION

The date palm, a dioecious monocotyledonous species, growths in the arid Middle East, northern Africa and the Sahara as far as Mauritania. It is characterized by a large genetic diversity, and represents the backbone of oasis agriculture and life in the desert. Today we are facing a severe decline of this diversity, measured but not detected by date palm growers and scientists. This loss is expressed by the scarcity or disappearance of cultivars. The causes of this genetic erosion are known: urbanization of palm groves, the market demands on a limited number of varieties, but are mainly due to diseases that are most destructive such as the Bayoud.

To compensate the damage caused by the Bayoud disease and losses of cultivars caused by traditional methods, regeneration of valuable varieties by culture techniques in vitro, using vegetative propagation which is extremely powerful, offers several advantages. Among the techniques developed, somatic embryogenesis is most common in the date palm. However, this technique is limited by losses during the germination of somatic embryos. To better understand the embryogenesis and overcome obstacles above, it is necessary to understand all important steps of zygotic embryogenesis.

Little information is currently available on the embryonic development of date palm. Thus, our present work might bring new information regarding the development of zygotic embryos of date palm which will contribute to improving the production of somatic embryos and to promote the mass propagation of elite date palm cultivars.

MATERIALS AND METHODS

Histological Study of Zygotic Embryos

The plant material used for the histological study is represented by female flowers 'Deglet nour' pollinated by traditional way by placing a spikelet male within the female inflorescence and young fruit. The flowers were harvested daily from the 1st day after pollination until day 21, and then every three days until the 96th day. The organs were immediately placed for 48 hours in a fixative mixture consisting of 25% glutaraldehyde, paraformaldehyde (10%) and caffeine (1%). After fixation, the flowers were carefully dissected, carpels isolated and dehydrated by successive rinsing in an increasing concentration of ethylalcohol for inclusion either in paraffin or resin in the LKB.

The organs included in paraffin were cut with a microtome "Leica", 7 µm thick. After deletion of paraffin and rehydration of sections, double staining, Periodic Acid-Schiff (P.A.S.) - Red dye polysaccharides - and Naphthol Blue Black - specific protein - was carried out. The organs included in the LKB resin were cut at 3 µm thickness with a microtome LKB Historange and underwent the same staining procedure.

The P.A.S. reaction to release aldehyde groups of polysaccharides with periodic acid, then stained by Schiff's reagent. Thus the walls and starch nature reserves appeared in red. Through Naphthol Blue Black, the protein reserves are blue black, nuclei and nucleoli in blue green.

Extraction and Electrophoresis of Total Proteins on Polyacrylamide Gel

Mature zygotic embryos and endosperm fragments of two cultivars, 'Deglet Nour' and 'Ghars', underwent electrophoretic study of proteins by SDS-PAGE according to the technique of Laemmli (1970).

Mature zygotic embryos (0.1 g) and fragments of endosperm were ground in liquid nitrogen and pulverized and extracted in 1 ml of extraction buffer at pH 7.5 in the presence of a protease inhibitor (PMSF). The extracts were centrifuged for 30 min at 10,000 g and 4°C. Protein levels contained in the supernatant were estimated by Bradford assay (1976), and the DO is read at 590 nm. Proteins were fractionated instead of revealed by gel electrophoresis separation of acrylamide, and visualization of bands was performed by Coomassie blue G250. The molecular weight of soluble proteins was assessed by comparison with the migration of protein molecular weight using known standards. It was determined according to their migration distances.

RESULTS

Histological Transformation of the Fertilized Ovule

Before fertilization, the mature ovule was surrounded by two integuments, outer and inner limiting a large embryo sac. The outer thick and vascularized integument was fully welded to the funiculus. The inner integument was thinner and totally invaded by polyphenols (Fig. 1).

Seven days after pollination, the cells micropylar apex of inner integument and some inner cells of the outer integument degenerated, while the outer integument cells enlarged and their walls became rigid due to thickening of their lateral surfaces. During ovule development, cells invaded the inner integument of polyphenols completely degenerated, and the outer integument, which became the seed coat of future seed.

At maturity the embryo sac, nucellar cells, degenerate and there persists a seat in the nucellar region of the micropyle (Fig. 2). It is thus a bag said tenuinucellé (Bouguedoura, 1991). Lysis, gradual persistent nucellar cells, was fully completed in the first division of the egg cell.

Evolution of Embryo Sac

The embryo sac of date palm Polygonum type contained four mature cells following the early degeneration of the three antipods. The embryo sac contains two synergid cells, an egg cell and a large central cell which is the future first endosperm cell (Fig. 2).

During development of the fertilized ovule in young seed, the embryo sac undergoes several transformations:

- Within the embryo sac, the synergid of the gametic complex degenerates after fertilization of the egg cell.
- Fertilization of the central cell with the second sperm nucleus takes place before fertilization of the egg cell and leads to the formation of the endosperm cell dense core fingered in outline. The latter undergoes mitosis forming a successive frame coenocytique nuclei endosperm. The endosperm of the date palm is first all nuclear, then gradually cellularized (Fig. 3). The endosperm, initially very rich in protein, is then enriched with polysaccharides to the origin of mannans. In the mature stage, the cell wall of the endosperm becomes very thick.

Embryo Development

The histological changes leading to the formation of the zygotic embryo begins at fertilization of oosphere. For the cultivars studied, fertilization of oosphere with the sperm nucleus takes place 5 days (d5) after pollination. The zygote became spherical and larger. At d8, its organization showed no structural peculiarity, it is hardly any different from that of a meristematic cell vacuole by its relatively developed. The partition between the egg cell was not of the same thickness on all sides. It was very thin, seemingly embattled in its apex facing the endosperm where it is evenly broken in some places (Fig. 4).

At d12, the zygote undergoes a first transverse division resulting in two overlapping and unequal cells, one apical (inner side of the bag) and one basal (micropylar side) elongated and larger. The whole of two cells was entirely limited by the cellulose wall of the egg cell (Fig. 5). The two cells are characterized by a large vacuole rejecting the nucleus and the cytoplasm against the cellulose wall.

Nineteen days (d19) after pollination, the apical cell undergoes mitotic divisions that lead to the formation of a proembryon which was surrounded by a thick wall (Fig. 6). The globule proembryonnaire continues slow segmentation by successive divisions to give the d61 globular embryo (Fig. 7). It is then bordered by a protoderme which consists of a layer of meristematic cells, isolating the embryonic tissues of endosperm.

Beyond the globular stage, an intense meristematic activity of embryonic cells can build a heart-shaped embryo with bilateral symmetry and that at d68 (Fig. 8).

This character reminds the classic structure of the embryo of dicotyledons.

However, in the embryo hickory date palm, the two territories are lateral to the establishment of the cotyledonary leaf and its sheath.

A space is created in stages between the embryo and endosperm, indicating that the embryo develops more likely at the expense of the endosperm. This presents an early stage of tissue structure associated with cell differentiation further. During early development stages of cotyledon (d75), shoot meristem of the first lateral position is being established within a cavity formed by the cotyledon (Fig. 9).

At d80, the embryo was bipolar, with a proximal meristem and distal pole corresponding to the smaller root. The cotyledon develops in the transverse beams and differentiates parallel provasculars. During embryonic development the apical meristem became functional by providing the first leaf primordium that will grow to a leaf cotyledonary post during germination (Fig. 10).

The central part of the cotyledon elongated then in the proximal direction forming a cotyledonary blade that removes the gemmule cotyledon. Three months after fertilization, the embryo developed with the production by the apical meristem of the second leaf primordium. Provascular beams are narrow and extend along the embryonic limb. Their number increased gradually as one approaches the proximal cotyledonary summit, their subsequent ramifications. The embryo at this stage has the same cylindrical appearance as the mature embryo (Fig. 11). The embryonic axis then formed a shoot meristem surrounded by two rough leaves, a very short hypocotyl axis traveled procambial beams and a very small root meristem.

In terms of reserves, they are essentially protein whose amount increased during the ontogeny of the embryo. However, we did not observe any clear distinction concerning the quantity of proteins between the apical and basal pole during embryonic development.

Analysis of Proteins by Electrophoresis Zygotic Embryos

In the seeds of palm trees, stored reserves were present in the small cotyledon embryo and endosperm in the massive and hard.

The seeds of palm trees undergo significant changes during their development and reserves are accumulated as protein (Morcillo et al., 1998), lipids (Ferdinando et al., 1985) and sucrose.

In the present article, a comparative study between protein zygotic embryos and endosperm of two varieties ‘Deglet Nour’ and ‘Ghars’ date palm is reported.

The electrophoretic study revealed several protein bands, whose molecular weight established for the four protein profiles is between 12 and 91 kDa (Fig. 12).

Analysis of protein profiles obtained showed that the proteins of endosperm and mature zygotic embryos show no major changes for the two varieties of date palms studied. These similarities in protein profiles suggest that storage proteins are the same in both compartments. In addition, this study has revealed a wealth of protein reserves of zygotic embryo and endosperm of date palm with the presence of three major proteins’ dense high molecular weight between 74 and 91 kDa.

DISCUSSION

The zygote of date palm is not elongated but it presents a discontinuous basal wall, as is reported in angiosperms (Russell, 1993).

The development of zygotic embryos of date palm is similar to what has been reported on coconut (*Coccos nucifera* L.) (Haccius and Philip, 1979). Like the date palm, the first division of the zygote is transverse and asymmetric, separating oosphere fertilized in a small apical cell and a large basal cell. The asymmetric nature of this division is important for further embryonic development (Shevell et al., 1994).

As at the date palm, mature zygotic embryos of coconut have a shoot meristem in lateral position (Haccius and Philip, 1979).

Our histological study revealed a structural homology between zygotic embryos of date palm and those of palm oil in the mature stage (Kamnoon and Preamrudee, 1999;

Aberlenc-Bertossi, 2001). Indeed, the work of Ferdinando et al. (1985) on palm oil indicates that the final appearance of the embryo is visible before the mature stage. However, according to Aberlenc-Bertossi (2001), in palm oil, the embryonic axis is more pronounced because it has produced several very young leaves.

Comparison of electrophoretic profiles of proteins of date palm and other palm species shows bands majority municipalities. So with oil palm, we observe three bands majority with common high molecular weight. However, in the palm oil these three protein bands are between 45 and 66 kDa (Morcillo, 1998).

Moreover, our results are consistent with those reported by Chandra Sekhar and DeMason (1988a) and Sghaier et al. (2006) who showed the presence of three major bands with high molecular weight in different varieties of date palm. Sghaier et al. (2006) revealed the presence of these proteins between 55 and 82 kDa.

In general, electrophoretic analysis revealed that the protein profiles of zygotic embryos and abumen date palm show great similarities with those found in oil palm (*Elaeis guinneensis* Jacq.), the Washingtonia (*Washingtonia filifera*) and coconut (*Coccos nucifera* L.) (Morcillo, 1998; Chandra Sekhar and DeMason, 1988b; DeMason and Chandra Sekhar, 1990). This suggests similarities in their protein composition of reserves.

The storage proteins of dicotyledonous are mainly albumins and globulins, while prolamins and glutelin are the major proteins in monocots (Derbyshire et al., 1976). The work of Sghaier et al. (2007) reveals that glutelin can be used as a biochemical marker for the late development of zygotic embryos of date palm. Studies from Morcillo (1998) on palm oil showed that proteins of low molecular weight were identified to 2S albumin, and those with high molecular weight globulins 7S. Morcillo (1998) showed that the 7S globulin type, which is the major storage protein in the palm oil can be used as markers of maturation in zygotic embryos of oil palm.

CONCLUSIONS

Zygotic embryogenesis is a key stage in the life of embryophyta. It starts from the double fertilization and determines the overall organization of the future plant.

In the date palm, the zygotic embryogenesis results in a histocytological and biochemical transformation of oosphere following fertilization. On the structural level, the embryogenesis is rather comparable to published data relating mainly to dicotyledonous. The study of the embryogenesis of monocotyls is less controlled and that of the palm trees is rather particular (Vallade, 1999).

During the first three months after pollination, growth of the embryo is slow. This period is also devoted to processing tissue of the ovule:

- First, there is degeneration of nucellar persistent cells, and the two synergid while the opposite disappears soon. The merger of the central cell with the second sperm nucleus giving the endosperm nucleus takes place before fertilization of oosphere. This asynchrony after the double fertilization is a common phenomenon in dicotyledons (Ducreux, 2002). The albumen is gradually shifting from stage to stage nuclear cell as in most plant species (DeMason et al., 1983).
- The egg cell or zygote undergoes an asymmetric first division and cross giving rise to two unequal cells. The early cleavage gives rise to a globular proembryon two months after pollination.
- During this study, we found that the date palm is one of the exceptions with a heart-shaped stage characteristic of dicotyledons. But, the lateral territories contribute to the overall construction of the cotyledonary leaf and sheath cotyledonary.
- Beyond the heart-shaped stage, the embryonic mitotic activity is fast and it promotes the development of the cotyledon sheathing and production of two young leaves. Althought, the palm tree embryo has the caracteristic to function before germination. After another month of development the final shape and size of the normal embryo are affected.
- The histology of the mature embryo stage showing the distinction of two parts, one part corresponding to the proximal cotyledon traversed by procambial beams and

surrounding the plumule and another distal part where are the axis hypocotyl and radicle; the latter beeing difficult to distinguish.

- With the date palm, the work of biochemistry is oriented towards the identification of protein accumulation in zygotic embryos, in order to improve the late phases of somatic embryogenesis and plant vigor regenerated from somatic embryos.

Our biochemical study showed accumulation of proteins in zygotic embryos and endosperm. The somatic embryo differs from the zygotic embryo in the absence of endosperm which is required to monitor germination (Brownfield et al., 2007).

Thus, germination and viability of somatic embryos depends on the establishment of protein reserves and especially the drivers of the beams that connect the part meristem of the root.

The plants from the germination of zygotic embryos are generally more vigorous than those from somatic embryos. This relative lack of effect may be due to incomplete maturation of somatic embryos (Crouch, 1982).

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Figures

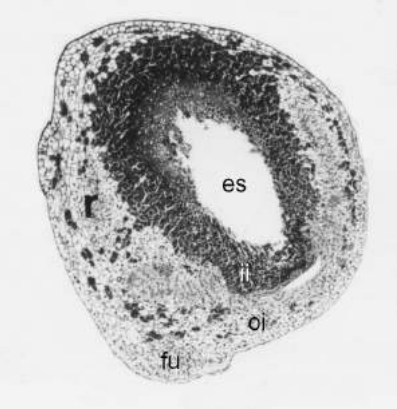

Fig. 1. Longitudinal section of the ovule before the fertilization. es, embryo sac; fu, funiculus; ii, inner integument; oi, outer integument. Scale bar: 315 µm.

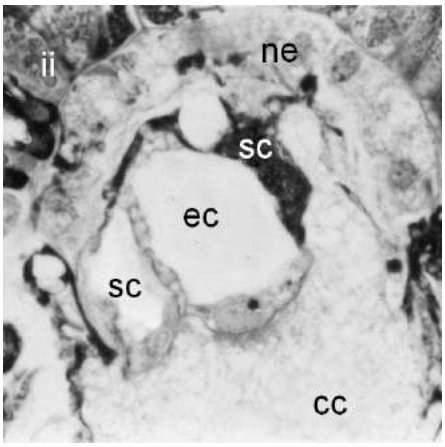

Fig. 2. Summit of the mature embryo sac. cc, central cell; ec, egg cell; ii, inner integument; ne, nucellar epidermis; sc, synergid cell. Scale bar: 20 µm.

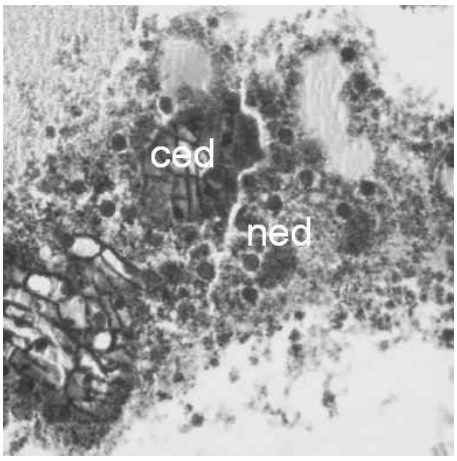

Fig. 3. Progressive cellularisation of some nucleus albumen. ced, cellular endosperm; ned, nuclear endosperm. Scale bar: 40 µm.

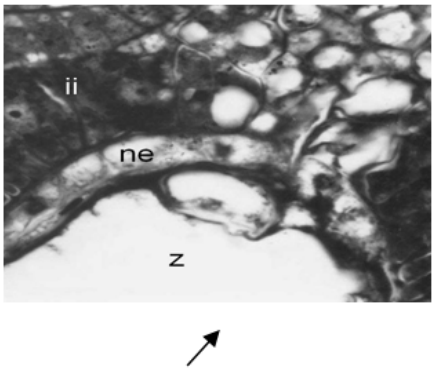

Fig. 4. The zygote to D8 showing a carved wall. ii, inner integument; ne, nucellar epidermis; z, zygote. Scale bar: 80 µm.

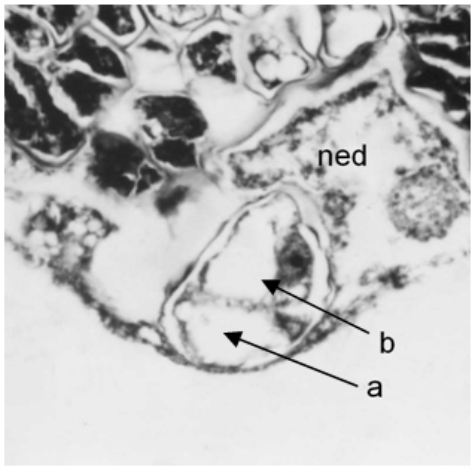

Fig. 5. Division of the egg cell. Presence of the apical cell, the future embryo and the basale cell future suspensor. a, apical daughter of the zygote of zygote; b, basal daughter of the zygote of zygote; ned, nuclear endosperm. Scale bar: 20µm.

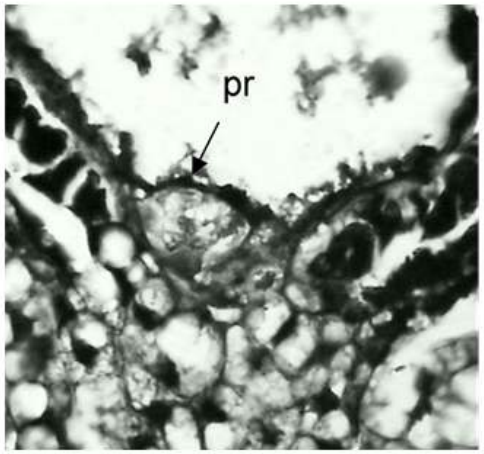

Fig. 6. Proembryo to D19. pr, pro embryo. Scale bar: 12 µm.

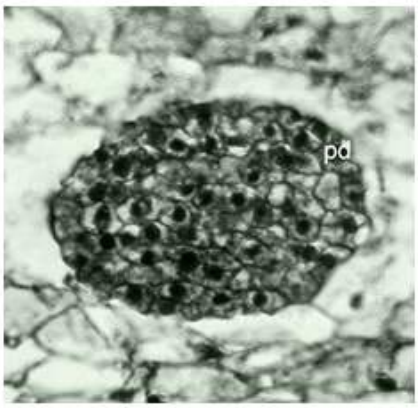

Fig. 7. Globular embryo to D61. pd, protoderm. Scale bar: 24 µm.

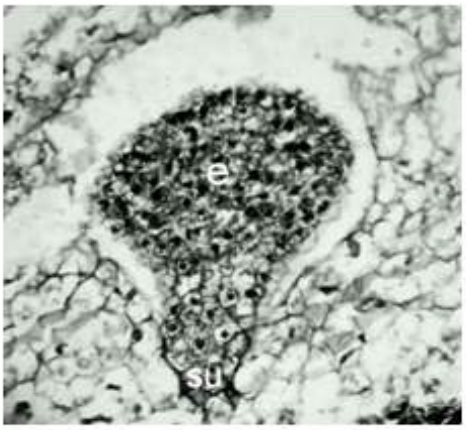

Fig. 8. Embryo at the stage cordiforme to D68. e, embryo; su, suspensor. Scale bar: 40 µm.

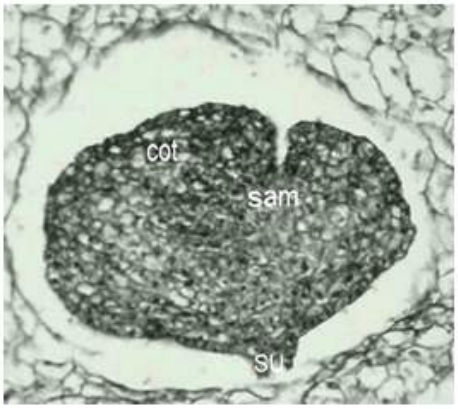

Fig. 9. General aspect of the embryo to D75. cot, cotyledon; sam, shoot apical meristem; su, suspensor. Scale bar: 56 µm.

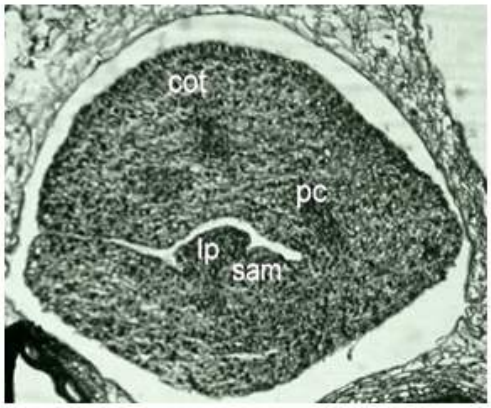

Fig. 10. General aspect of the embryo to D80. Installation of the apical meristem and the development of the cotyledon. cot, cotyledon; lp, leaf primordia; pc, procambium; sam, shoot apical meristem. Scale bar: 100 µm.

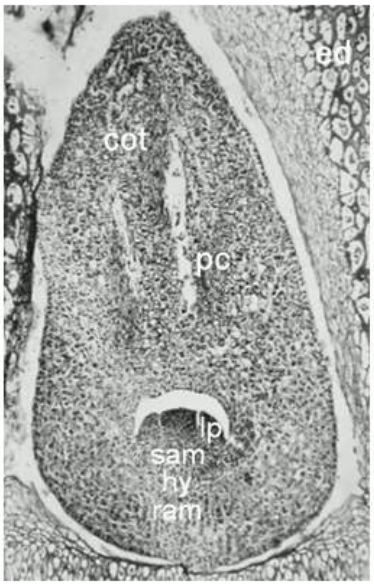

Fig. 11. Histological aspect of the zygotic embryo to J96. Scale bar: 70 µm. cot, cotyledon; ed, endosperm; hy, hypophysis; lp, leaf primordia; pc, procambium; ram, root apical meristem; sam, shoot apical meristem.

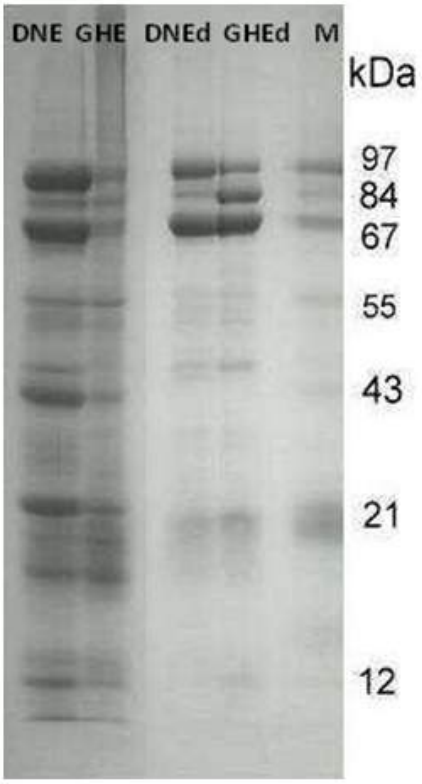

Fig. 12. Electrophoretic profiles of the total proteins of the albumen and the zygotic embryos of both varieties of palm date (‘Deglet nour’ and ‘Ghars’). DNE, zygotic embryo of the variety ‘Deglet nour’; GHE, zygotic embryo of the variety ‘Ghars’, DNEd, endosperm of the variety ‘Deglet nour’; GHEd, endosperm of the variety ‘Ghars’.

In Vitro Culture Techniques for Conservation of Date Palm Germplasmin in Arab Countries

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Keywords: date palm, in vitro, germplasm preservation

Abstract

Great numbers of date palm trees have been lost in Arab countries due to diseases (Morocco), destruction of habitats (Egypt) and wars (Iraq). Expansion of date palm plantations and replanting trees to compensate loss caused by diseases or man-made factors are restricted due to lack of adequate propagation and conservation methods. The sexual propagation method cannot be used commercially for propagation of the cultivars of interest in a true-to-type manner. In the vegetative conventional propagation, few numbers of offshoots are produced from the mothers in their juvenile life cycle. Moreover, conservation of date palm germplasms is difficult-to-store in the form of offshoots or in field collections. Tissue culture and molecular biology techniques have great potential for the collecting, multiplication and storage of date palm germplasm. In vitro storage methods have been developed for preservation of date palm germplasm which can be used extensively for the international exchange of germplasm because of their obvious advantages over in vivo material, notably their reduced weight and volume, and phytosanitary conditions. Moreover, plant material can thus be multiplied, stored and exchanged in a disease-free state. The results of our studies indicate that short and mid-term periods of storage of date palm tissue cultures were recognized by controlling of environmental growth conditions and nutrient media composition. Long-term storage has been achieved by in vitro cryopreservation. Moreover, encapsulation of somatic embryos in alginate beads has been recognized recently as a possible way of germplasm exchange in date palm varieties. Otherwise, molecular biology techniques and their uses in the conservation, evaluation and utilization of genetic resources have been used for characterization and detection of genetic stability of the conserved date palm tissue cultures. This article presents the methods and aspects of plant biotechnology used for management and conservation of date palm germplasms.

INTRODUCTION

Cultivation of date palm plant in different ecosystems around the world resulted in many local varieties and cultivars that represent genetic diversity. Advanced (current and obsolete) cultivars are resources, which may have importance to future breeders and these need to be preserved**.** Like many other plants, date palm has been threatened by human intervention and exploitation. Countries which hold significant amounts of date palm diversity have a responsibility unto themselves as well as to humanity at large to safeguard such diversity and make it possible to be utilized for the development of their own countries as well as others. The common method used to preserve the genetic resources of vegetative propagated plants such as date palm is as whole plants in the field which has serious problems with a field gene bank. A field gene bank has traditionally been used for perennial plants, including species that have a long life cycle to generate breeding and/or planting material. The main disadvantage of this method is that the material is susceptible to pests, diseases and vandalism. In addition, distribution and

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exchange from a field gene bank are difficult because of the vegetative nature of the material and the greater risk of disease transfer. Moreover, conservation of date palm genetic diversity is imperative due to its high heterozygosity and it is therefore of limited interest for conservation by conventional means.

Biotechnology continues to have a key role in the conservation and sustainable utilization of all types of biodiversity. Biotechnology techniques offer an alternative method for conservation of such plant material. The potential of in vitro culture methods for the conservation of the genetic resources of vegetatively propagated crop species was recently recognized. Two types of in vitro gene banks for conservation have been reported. The in vitro active genebank where cultures are maintained under slow growth and the in vitro base gene bank where cultures are cryopreserved (Withers and Williams, 1985). Slow growth is achieved by modifying the culture medium or reducing temperature requirements (Withers, 1991). One of the principle long-term in vitro conservation methods is cryostorage. Cryopreservation is generally understood as storage between -79 and -196°C, the low extreme being the temperature of liquid nitrogen. The major advantage of plant material at such temperature is that both metabolic process and biological deterioration are considerably slowed or even halted (Kartha, 1981). In addition, it is believed that cryopreserved material remains genetically stable, thus affording an advantage over conventional conservation methods (Withers, 1980, 1983). Molecular biology techniques and their uses in the conservation, evaluation and utilization of genetic resources can be summarized in: a) comparisons between collection or population and measurement of genetic diversity before and after storage and b) detection and characterization of collections. Tissue culture and molecular biology techniques have great potential for the collecting, multiplication and storage of date palm germplasm. Date palm germplasm has been preserved in vitro in the form of shoot tips, somatic embryos, pollen, callus and cell suspension cultures (Tisserat, 1981; Tisserat et al., 1985; Mater, 1987; Bekheet et al., 2001, 2005, 2007; N´Nan et al., 2008). The miniaturization of explants allows reduction in space requirements. Disease-free stock is simplifying quarantine procedures for the regional and international exchange of germplasm. Moreover, molecular and biochemical analyses have been investigated for characterization of the conserved samples (Salman et al., 1988; Bekheet et al., 2007; Othmani et al., 2009). This article discusses different aspects and applicable methods of biotechnology used for in vitro conservation of different varieties of date palm.

DISCUSSION

Importance of Conservation and Expected Impacts

The date palm tree has a great socioeconomic importance and nutritional value in different regions of the world, especially the Middle East and North Africa. In addition, the palm tree tolerates adverse environmental conditions and it is important in reducing desertification. The world total number of date palms is about 100 million, distributed in 30 countries, and producing between 2.5 and 4 million tons of fruit per year. The top five date producing countries are Egypt, Iran, Saudi Arabia, Pakistan and Iraq, accounting for about 69% of total production. If the next five most important countries are included, i.e., Algeria, United Arab Emirates, Sudan, Oman and Morocco, then this percentage rises to 90%. Like many other plants the date palm has been threatened by human intervention and exploitation. Land use planning which includes construction and building of roads, factories, canals, dams and new residential areas. In this respect, In Egypt, date palm trees decreased from 2.5 millions to 1,061,189 in Aswan area due to the build up of the High Dam (Hussein, 1993). Otherwise, biotic factors such as pests and diseases can attack date palm plants resulting in negative impacts on the genetic variability within the species. In this connection, Bayoud disease has destroyed more than twelve million palms in Morocco and three million in Algeria. Bayoud destroyed the world's most renowned varieties that are susceptible to the disease and particularly those which produce high quality and quantity fruit ('Medjool', 'Deglet Nour', 'Bou Fegouss'). Otherwise, a lot of

diversity of date palm losses are caused directly by war or indirectly by destruction of habitats. However, during the Gulf and Iran-Iraq wars, many palm trees were destroyed and more died when the southern marshes were drained. For instance, the population of 16 million date trees around Basra before the wars was reduced to around 3 million in 2003 (MacFarquhar, 2003). Progress in the field of breeding, genetics, crop improvement, and expansion of commercial plantings of date palm has been restricted by the habit and long-lived nature of these monocotyledonous trees. Without substantial replanting, the date palm orchards will continue to decline as productive farms and will correspondingly decline as places of aesthetic quality. There is a great need to put mechanisms for protection and conservation of date palm cultivars in the Arab region. The rapid propagation of date palm by the vegetative traditional mean is impossible due to the limited number of offshoots produced and the fact that offshoot production is limited to a certain period in the palm's life span. Biotechnology can lead to a substantial increase in value of natural resources. With a balanced approach and careful planning, this technology can be used for conservation and distribution of date palm and can contribute towards sustainable development in Arab countries. Now, the healthy and vigorous plants of the superior cultivars have been produced by tissue culture for large scale production. Moreover, the application of different methodologies of biotechnology to germplasm storage can be used for conservation of date palm varieties. The use of germplasm in plant breeding leads to changes in crop output and breeder's requests represent the demand for germplasm in terms of crop in production and trade. The conservation and distribution of superior date palm cultivars have economical and social impacts such as a) improving living conditions of the farmers by increasing net returns from production and b) increasing job opportunities for the people by development and promotion of small-scale agro-industries. Moreover, important ecological impacts can be gained by protection and preservation of date palm varieties in Arab countries. The palm tree successfully tolerates extremely adverse environmental conditions, including drought, high temperature and salinity, which are the peculiar criteria of desert lands. It makes a significant contribution towards the creation of equable microclimates within the fragile oasis ecosystems, thus enabling sustainable agricultural development in many drought and saline affected regions. Moreover, cultivation of date palm does not require any agrochemical that may release toxic components into the environment, and as a result, does not affect the health of the soil.

In Vitro Storage

Date palm germplasm is traditionally conserved in the form of field collections of trees in its cultivation areas. However, because of disease and insect problems, weather factors and improper management, many collections may be at risk, and germplasm may be lost. Moreover, germplasm cannot be exchanged or handled easily using the conventional means. Plant in vitro technology offers a potential solution for conservation of such germplasm. In vitro conservation methods are employed depending on the storage duration required. For short- and medium-term storage, various techniques have been devised that allow reduction of growth and increase the intervals between subcultures. In vitro conservation techniques using slow growth storage have been developed for date palm. In this respect, Bekheet et al. (2001) described a method for preservation of Egyptian date palm ('Zaghloul') tissue cultures by slow growth. Shoot buds and callus cultures were successfully stored for 12 months at 5ºC in the dark (Fig. 1). In these conditions, high percentages of the cultures remained viable without serious signs of senescence. Moreover, the role of sorbitol as osmotic stress agent in in vitro storage of date palm cultures was examined. In normal growth conditions, healthy shoot bud cultures were obtained after 6 months of storage on medium containing 40 mg/L sorbitol. However, this period extended for 9 months in case of callus cultures (Fig. 2).

For long term storage, cryopreservation, that is storage at ultra-low temperature, usually that of liquid nitrogen (-196°C), is employed. At this temperature, all cellular divisions and metabolic processes are stopped. The plant material can thus be stored

without alteration or modification for a theoretically unlimited period of time. Moreover, cultures are stored in a small volume, are protected field and will facilitate collecting and improve its efficiency. Earlier reports have described storage methods of date palm tissue cultures by cryopreservation (Tisserat, 1981; Ulrich et al., 1982; Tisserat et al., 1985; Mater, 1987; Towill et al., 1989; Bagnoil et al., 1992; Mycock et al., 1995, 1997). Recently, an applicable method for in vitro cryopreservation of date palm has been developed by Bekheet et al. (2007). Undifferentiated tissue cultures (nodular cultures) were successfully cryopreserved by freezing methods and subsequently regenerated plants. The potential of dehydration caused by air drying to cryopreservation of date palm tissue cultures through direct immersion in liquid nitrogen was subjected. Among different types of sugars (fructose, glucose, sorbitol and sucrose) used as osmotic agents in preculture medium, sucrose was the best for the survival of cryopreserved date palm tissue cultures. To determine the potential of vitrification on freezing tolerance, cultures were exposed to a vitrification solution (22% (w/v) glycerol, 15% (w/v) ethylene glycol, 15% (w/v) propylene glycol and 7% (w/v) dimethylsulfoxide (DMSO)) for 20-100 min. Cultures were kept in liquid nitrogen (-196°C) for 48h. The maximum rate of survival was obtained with cultures exposed for 80 min at 0°C followed by 40 min at 25°C.

Synthetic Seeds

The establishment of synthetic seeds has multiple advantages including ease of handling, potential long-term storage and low cost of production and subsequent propagation. Although a variety of natural and synthetic polymers are available for encapsulation, sodium alginate is the most commonly used gel-matrix because of its easy gelling properties, non-toxicity and low cost. In this respect, a reliable system for preservation of date palm germplasm via artificial seeds was recognized by Bekheet et al. (2005). Somatic embryos proliferated in vitro from shoot-tip cultures were encased in sodium alginate capsules and stored for twelve months. Somatic embryos in four maturation stages, i.e., globular, torpedo, cotyledon and late cotyledon were taken and dried on a laminar flow bench and then mixed with gel of 3% sodium alginate prepared in distilled water. An antibiotic mixture contained rifampicin (60 mg), cefatoxine (250 mg) and tetracycline HCl was used to avoid contamination. The embryos were placed into calcium chloride solution (2.5%) for 30 min and then stored at 5 and 25ºC. After 12 months, the encapsulated embryos were taken and sown in distilled water and then cultured on MS-hormone-free medium. The cotyledon stage of embryos maturation and 3% of sodium alginate used as a gel matrix were the best for viability and conversion to plantlets. For plantlets recovery and development from encapsulated somatic embryos, 20 g/L of sucrose was added to the culture medium. The observations indicated that the highest percentage of viability was recorded with the cotyledon stage. However, the highest percentage of conversion to plantlets was registered with the late cotyledon stage.

Monitoring Genetic Stability

The applications of date palm characterization include elucidating systematic relationships between accessions; assessing gaps and redundancies in the collection; development of core subsets; characterizing newly acquired germplasm; maintaining trueness-to-type; monitoring shifts in population genetic structure in heterogeneous germplasm; monitoring genetic shifts caused by differential viability in storage or in vitro culture; exploiting associations among traits of interest and genetic markers; and genetic enhancement (Bretting and Widrlechner, 1995). Stability of the preserved date palm plant materials has been observed by Ulrich et al. (1982). They reported that date palm callus was frozen to -196°C, thawed, and then examined in terms of subsequent growth and plantlet generation. Isozyme analysis of leaves from regenerated plantlets was performed to detect possible genetic alterations caused by the cryogenic treatments. The isozyme patterns of extracts of triplicate leaf samples from each treatment were examined for enzyme polymorphism and compared with each other and with samples taken as controls, from several field-grown date palm cultivars. The obtained results indicate that within the

'Medjool' cultivar no enzyme differences were expressed among the leaflets of plantlets from untreated, PGD-treated, and frozen cultures. Salman et al. (1988) analyzed palm plants from tissue cultures for their isozyme polymorphism and chromosome number. They found that all regenerated plants had the basic chromosome numbers of 2n=36 except one of 2n=70. An histo-cytological study was performed on apices of in vitro plantlets of date palm submitted to a cryopreservation process (Bagniol et al., 1992). The apices submitted to the cryopreservation process were sampled on in vitro plantlets of date palm (*Phoenix daetyrifeera* L. 'Bou Sthammi Noir'). After freezing in liquid nitrogen, the apices showed cellular heterogeneity. Some cells conserved their meristematic characters. This was the case in the cellular layers corresponding to the meristem itself, whereas in the underlying zone, where the cells were more vacuolated, some were damaged, showing broken cell walls were observed in some samples. During culture - prior to the cryoprotective treatment, obvious starch synthesis occurred in some cells of the samples. Recently, Randomly Amplified Polymorphic DNA (RAPD) analysis was used to examine the genetic stability of cryostored date palm tissue cultures (Bekheet et al., 2007). Both treated and non-treated date palm tissue cultures in addition of field grown plants were identical to each other with primers used.

Future Strategies

The importance of germplasm conservation is being increasingly realized by many developing countries. There is a need for extensive government cooperation and funding in Arab countries to protect and preserve date palm germplasm. This should include: a) participation in regional and national activities related to both in vitro and in vivo conservation, b) establish links with the National Bureau of Plant Genetic Resources (NBPGR) for management of plant genetic resources related to horticulture crops. Most superior date palm cultivars need to be conserved at three broad levels i.e., ecosystem, genotype and gene levels. The conventional methods used to conserve date palm varieties should be supplemented by rapid developments in plant biotechnology. Interventions at cellular and molecular levels became critical factors in germplasm collection and conservation of date palm. There is much that can be done using biotechnology techniques not only to conserve germplasm of this important species but also to develop many new cultivars. Tissue culture techniques offer the opportunity for in vitro collecting, rapid propagation of medium and long-term storage of germplasm and its distribution. These techniques are invaluable to complement other health and conservation strategies, particularly for superior and rare cultivars. Moreover, DNA banks provide novel options for date palm genebanks. In response of the World Trade Organization (WTO) related obligation, the development of strong and effective farmers' rights is of increasing importance. This should allow to defend their interests against fraudulent appropriation and to allow them to benefit from their own knowledge in legal and commercial sense. Management of intellectual property protection of plant materials will encourage date palm breeders to protect and develop their valuable varieties.

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Figures

Fig. 1. Shoot buds of date palm (‘Zaghloul’) stored for 12 months at 5ºC in the dark.

Fig. 2. Callus culture of date palm (‘Zaghloul’) stored for 9 months on medium containing 40 mg/L sorbitolat in normal growth conditions.

Effect of Salt Stress and Proline on Chemical Content of Embryogenic Callus and Somatic Embryos of Date Palm (*Phoenix dactylifera* L. 'Ashkar')

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Keywords: carbohydrates, in vitro, micropropagation, osmoregulation, protein

Abstract

The objective of the current study is to find out the effect of salt stress as sodium chloride and proline in the culture media on total soluble carbohydrates, proteins, and proline content of embryogenic callus and somatic embryos of date palm 'Ashkar'. Quarters of shoot tips were cultured on MS medium supplemented with NAA (30 mg/L) and 2-ip (3 mg/L) for callus initiation and multiplication. NAA was reduced to 1 mg/L and 2-ip to 0.1 mg/L for the development of somatic embryos. Sodium chloride was added at 0, 0.5, 1, 1.5 and 2% and proline at 0, 25, 50, 75 and 100 mg/L to the media of well developed callus and somatic embryos and the interaction between the two factors was also evaluated. Results showed that an increase of sodium chloride concentration in the medium led to a decrease of total soluble carbohydrates and proteins content of embryogenic callus and somatic embryos, however it increased free proline. When proline was added to the culture media, it increased carbohydrates, proteins, and free proline in both embryogenic callus and somatic embryos. The interaction between sodium chloride and proline in the culture media had a positive significant effect on all studied chemical contents of embryogenic callus and somatic embryos. It was concluded that salt stress caused a negative impact on date palm callus and somatic embryos and those effects could be decreased by the addition of proline which significantly improves carbohydrates and proteins content. This might also help in the selection of high salt resistant callus cells and regeneration of date palm plantlets growing well under high salt stress condition.

INTRODUCTION

Date palm has been classified as a salt resistant fruit tree, however, the productivity of date palm decreases with the increase of salts in soil or irrigation water. The reduction in productivity starts when the salt concentration is 0.288% and there is no production of date fruits when salts reach 1.48% (Hassan, 1991). Salinity has a negative effect on growth and development of plants through osmotic stress and the toxic effect of sodium and chloride ions, besides the ionic imbalance that is induced by accumulation of those ions (Sairam and Tyagi, 2004). The attempts to improve salt resistance by traditional plant breeding gave a limited success because salt resistance is a complex physiological and genetical feature (Flowers, 2003). The tissue culture technique is considered a suitable system to produce plant strains resistant to salt stress through callus culture which is used for many plants (Miles, 1991; Vitaglinoet et al., 1992; Wanas et al., 1999).

The objective of the present study was to find out the effect of salt stress and proline on the chemical content of embryogenic callus and somatic embryos of date palm 'Ashkar' grown under different levels of sodium chloride and how it interacts with the addition of proline to the culture media.

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MATERIALS AND METHODS

Two-year-old offshoots of date palm 'Ashkar' were used as a source of shoot tips explants. Each shoot tip was divided into four equal quarters and surface sterilized by 20% commercial bleach then rinsed three times by sterile distilled water. Explants were cultured on MS medium (Murashigie and Skoog, 1962) supplemented with naphthaleneacetic acid (NAA) (30 mg/L) and 2-isopentenyl adenine (2-ip) for callus induction (Jasim, 2000) and multiplication. The NAA concentration was reduced to 1 mg/L and 2-ip to 0.1 mg/L to enhance the production of somatic embryos from embryogenic callus. Well developed embryogenic callus and somatic embryos were cultured on different levels of sodium chloride (NaCl) in the culture media (0, 0.5, 1, 1.5 and 2%) and proline levels were 0, 25, 50, 75 and 100 mg/L besides the interaction between high NaCl concentration (1.5 and 2%) and the same proline concentrations in the culture media. Chemical analyses were done for embryogenic callus and somatic embryos which include the following calculations.

Total Soluble Carbohydrates

The method of phenol-sulfuric acid used to measure soluble carbohydrates as stated by Herbert et al. (1971). A spectrophotometer was used to measure absorption spectrum at a wave length of 488 nm and a standard curve of glucose was made for calculation.

Proteins

The micro Kjeldhal was used to measure proteins as proteinious nitrogen by steam distillation method (Page, 1982).

Proline

A photometric method of Troll and Lindsley (1955) was used. A spectrophotometer was used to read the absorption spectrum at a wave length of 490 nm.

Statistical analysis of the results was done for a factorial experiment with two factors in a completely randomized design (Snedicor and Cochran, 1980).

RESULTS AND DISCUSSION

Sodium Chloride Effect

Results presented in Tables 1 and 2 show the effect of sodium chloride on the chemical content of embryogenic callus and somatic embryos, respectively. A high concentration of sodium chloride (1.5 and 2%) decreased total soluble carbohydrates in both embryogenic callus and somatic embryos. The decrease in carbohydrates under salt stress condition might be related to the salty condition that makes cells spend more energy for osmoregulation to withstand the disturbance caused by salt accumulation inside cells. Also a high salt condition increases the respiration rate through the effect of sodium ions on the respiration cycle that leads to the decrease in carbohydrates (Maas, 1986; Wang et al., 1999; Huang and Liu, 2002).

Results also indicated that an increase in sodium chloride concentration in culture media led to a decrease in protein content in both embryogenic callus and somatic embryos especially under 2% of sodium chloride where it reached 4.53% compared to 7.74% under the control treatment for embryogenic callus and 6.08% compared to 8.21% under the control treatment for somatic embryos. The decrease in protein content under salt stress might be related to the inhibition of protein synthesis by high salt condition (Kaouthar et al., 2001). Also high salts in the culture media might inhibit absorption of necessary elements for protein synthesis such as nitrogen, besides the negative effect of salinity on the biosynthesis of mRNA which leads to the disturbance of protein synthesis (Witting and Wilson, 2003).

The proline content of embryogenic callus and somatic embryos were significantly affected by the increase in sodium chloride in the culture media. A proline content under

2% sodium chloride in the culture media increased by almost four times its concentration under the control treatment for embryogenic callus (Table 1) and by almost three times for somatic embryos (Table 2). The increase of free proline under salt stress may be due to the stimulation of its synthesis which is a useful defensive way of plants (Saliem, 2000). Also the increase of free proline is a result of osmotic disturbance within cells that leads to a decrease of osmotic potential and proline is synthesized in the cytoplasm of stressed cells which keep the equilibrium between vacuole and cytoplasm (Delauney and Verma, 1993). The accumulation of free proline in the callus of date palm 'Barhee' under high salt condition is also mentioned by Al-Khayri (2002).

Proline Effect

The exogenous addition of proline to the culture media caused an increase in total soluble carbohydrates in embryogenic callus which reached 41.86 mg/gm fresh weight when proline was added by 100 mg/L compared with the control treatment which was 17.03 mg/gm (Table 3). The same effect of proline was observed on somatic embryos (Table 4) where proline at 100 mg/L increased total soluble carbohydrates to 53.08 mg/gm fresh weight compared to 28.47 mg/gm under the control treatment. The increase of total soluble carbohydrates was due to the enhancement of proline to the metabolism that hydrolyzed insoluble carbohydrates to the soluble state (Trembly and Trembly, 1991).

The protein content of embryogenic callus and somatic embryos was also affected by the addition of proline to the culture media (Tables 3 and 4), respectively, where the highest protein content resulted from 100 mg/L proline for embryogenic callus and somatic embryos. The increase in proline concentration caused an increase in protein because proline is an essential amino acid that activates protein synthesis and increases its levels in callus and somatic embryos.

The addition of proline to the culture media increased the free proline within callus and somatic embryos and the highest free proline resulted from the treatment by 100 mg/L proline for both embryogenic callus and somatic embryos.

Interaction Effect between Sodium Chloride and Proline

The interaction between sodium chloride at 1.5% and proline at 100 mg/L had a significant effect on total soluble carbohydrates which were 26.58 and 39.92 mg/gm fresh weight in embryogenic callus and somatic embryos, respectively (Tables 5 and 6). The lowest total soluble carbohydrates resulted from the interaction between sodium chloride at 2% and 0 proline which was 4.35 mg/gm fresh weight of the embryogenic callus and 13.53 mg/gm fresh weight of somatic embryos. The interaction also had a significant effect on proline content, where it was at its highest concentration in both embryogenic callus and somatic embryos treated by sodium chloride at 2% and 100 mg/L proline in the culture media (Tables 5 and 6). Results also showed no significant interaction between sodium chloride and proline in their effect on protein content of embryogenic callus and somatic embryos. The accumulation of free proline in the embryogenic callus and somatic embryos in the culture media under high sodium chloride and high exogenous proline might be related to the synergistic effect of both of them. Proline plays an essential role in the osmoregulation of plant cells when it accumulates at high concentration at the cytoplasm and decreases water potential of cytoplasm and this will cause a balance with the low water potential of the vacuole resulting from the accumulation of ions in it and that will keep a suitable turgor of cells and grow under salt stress condition (Greenway and Munns, 1980). It could be concluded that high salt stress had negative effects on embryogenic callus and somatic embryos in the culture media and those effects can be decreased by proline which increased soluble carbohydrates and protein under salty conditions. This might help in the selection of high salt resistant callus cells and regeneration of salt resistant date palm plantlets.

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Tables

Table 1. Effect of sodium chloride on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of embryogenic callus of date palm 'Ashkar'[1].

NaCl %	Carbohydrates	Protein	Proline
0	13.51b	7.74a	0.16e
0.5	10.28c	6.71b	0.29d
1.0	17.48a	5.32c	0.48c
1.5	8.11d	5.02d	0.56b
2.0	4.35e	4.53e	0.59a

[1] Figures followed by the same letter are not significantly different (P=0.05).

Table2. Effect of sodium chloride on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of somatic embryos of date palm 'Ashkar'[1].

NaCl %	Carbohydrates	Protein	Proline
0	26.75b	8.21a	0.26e
0.5	22.24c	7.73b	0.34d
1.0	28.66a	7.03c	0.67c
1.5	18.62d	6.85d	0.70b
2.0	13.71e	6.08e	0.73a

[1] Figures followed by the same letter are not significantly different (P=0.05).

Table 3. Effect of proline on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of embryogenic callus of date palm 'Ashkar'[1].

Proline (mg/L)	Carbohydrates	Protein	Proline
0	17.03e	7.75e	0.16e
25	23.75d	7.79d	0.32d
50	29.94c	7.91c	0.57c
75	36.15b	8.08b	0.66b
100	41.86a	8.21a	0.70a

[1] Figures followed by the same letter are not significantly different (P=0.05).

Table 4. Effect of proline on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of somatic embryos of date palm 'Ashkar'[1].

Proline (mg/L)	Carbohydrates	Protein	Proline
0	28.47e	8.80e	0.26e
25	35.34d	8.89d	0.42d
50	41.86c	9.10c	0.68c
75	49.80b	9.19b	0.71b
100	53.08a	9.37a	0.77a

[1] Figures followed by the same letter are not significantly different (P=0.05).

Table 5. Effect of the interaction between sodium chloride and proline on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of embryogenic callus of date palm 'Ashkar'[1].

	NaCl (%)	Proline (mg/L)					Average NaCl effect
		0	25	50	75	100	
Carbohydrates	0	17.03j	23.75e	29.94c	36.15b	41.86a	29.74a
	1.5	8.11n	16.69k	19.62h	23.20f	26.58d	18.84h
	2	4.35o	10.81m	14.87l	19.13i	22.18g	14.27c
	Average proline effect	9.82c	17.08d	21.48c	26.16b	30.21a	
Protein	0	7.75a	7.79a	7.91a	8.08a	8.21a	7.94a
	1.5	5.02a	5.24a	6.27a	6.44a	6.73a	5.94h
	2	4.53a	4.67a	5.33a	5.71a	6.22a	5.29c
	Average proline effect	5.76e	5.90d	6.50c	6.74b	7.05a	
Free proline	0	0.16o	0.32n	0.56l	0.66i	0.70h	0.48c
	1.5	0.55m	0.68i	0.85f	0.89e	0.95c	0.78b
	2	0.59k	0.79g	0.92d	1.17b	1.69a	1.03a
	Average proline effect	0.43e	0.60d	0.78c	0.90b	1.11a	

[1] Figures followed by the same letter are not significantly different (P=0.05).

Table 6. Effect of the interaction between sodium chloride and proline on total soluble carbohydrate (mg/gm fresh weight), total protein (% dry weight) and proline (microgram/gm dry weight) of somatic embryos of date palm cv Ashkar*.

	NaCl(%)	Proline					Average NaCl effect
		0	25	50	75	100	
Carbohydrates	0	28.47i	35.34e	41.86c	49.80b	53.08a	41.71a
	1.5	18.61m	24.54k	28.65i	33.72g	39.92d	29.09b
	2	13.53n	18.96l	26.70j	31.81h	34.06f	25.01c
	Average proline effect	20.20d	26.28c	32.40b	38.44a	42.35a	
Protein	0	8.80a	8.89a	9.10a	9.19a	9.37a	9.06a
	1.5	6.85a	7.04a	8.31a	8.62a	8.85a	7.93b
	2	6.08a	6.28a	6.99a	7.47a	7.66a	6.90c
	Average proline effect	7.24a	7.40d	8.13c	8.42b	8.62a	
Free proline	0	0.26m	0.42i	0.68k	o.70j	0.77h	0.56c
	1.5	0.70j	0.77h	0.88f	0.98d	1.09c	0.88b
	2	0.73i	0.87g	0.93e	1.35b	1.90a	1.16a
	Average proline effect	0.56d	0.69c	0.83b	.01e1	1.25a	

[1] Figures followed by the same letter are not significantly different (P=0.05).

Investigation of Somatic Embryogenesis for In Vitro Cultures of Date Palm (*Phoenix dactylifera* L.)

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Keywords: *Phoenix dactylifera*, in vitro culture, growth regulators, sucrose

Abbreviations: 2,4-D: 2,4-Dichlorophenoxyacetic acid; 2-ip, 6.γ.γ.dimethylallylamino purine; NAA: naphthalene acetic acid.

Abstract

Date palm is increasingly recognised as an important crop in northern Nigeria. Agriculturally, it can sustainably generate incomes for farmers to take families out of the poverty line while at the same time it can be used as a tree crop to check desertification. To achieve these expectations, a national date palm development program involving planting material production and date palm agronomy is currently underway. The somatic embryogenesis method is being currently adopted to produce planting materials. Apical meristem and leaf explants were used to initiate in vitro cultures. A range of media and media conditions were tested and different media induced callus. These media were supplemented with different growth regulators, sucrose at different concentrations and nitrogen in the growth medium. NAA and 2,4-D provoked callus production. Callus could be produced at various sucrose concentration levels but 30 g/L was optimum. Callus generation potential was best from apical meristems, followed by leaf bases and leaves. Similarly different media also induced somatic embryos but the most reliable medium was the one containing 0.05 mg/L NAA and 1 mg/L 2-ip. The somatic embryos readily developed into shoots which could be rooted in NAA and sucrose (45-90 g/L). Some plants are in the nursery while some have been planted in the field undergoing observation.

INTRODUCTION

Date palm is important in the dry regions of northern Nigeria. It has been grown in the region for centuries and is adapted to the ecology of the region. It is an important component of the farming system in the area where it is planted in mixed cropping with arable crops such as maize, cowpea, sorghum, ground nut and millet. It is an important source of income as a few date palm trees provide significant cash earnings while the food crops provide for the dietary needs of the family. Current concerns on enhancing farmers' livelihoods within the context of the millennium development goals and checking desert encroachment in the area have renewed interest in date palm as one of the candidate crops with multiplier benefits that can be used quickly to ameliorate the living standards of the rural poor and at the same time, improve the environment. This project therefore fits into a national date palm development program for Nigeria with the goal of providing planting materials for distribution to farmers. Other components of the program include development of fruit processing techniques and marketing strategies.

In vitro propagation is very important as an option to complement conventional methods of generating planting materials. It is a viable method of producing large numbers of date palm planting materials. The advantages offered by in vitro multiplication include the avenue it provides of multiplying a single productive individual plant. For date palm which is dioecious, this feature is extremely useful. Many published findings are available on date palm in vitro multiplication through somatic embryogenesis

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(Tisserat, 1979; Sharma et al., 1984; Daquin and Letouze, 1988; Letouze et al., 2000) as well as by direct organogenesis (Rhiss et al., 1979; Beauchesne, 1982). However, the importance of the crop, the continuous expansion of cultivation areas into new communities and the preference sometimes, for particular local varieties make it necessary to evolve and refine working protocols. Our current efforts on date palm in vitro multiplication use the somatic embryogenesis method.

Different factors affect the performance of cultures in vitro. These factors include the type and concentration of plant growth regulators added to the basal medium as well as the interaction between auxins and cytokinins (Rao et al., 1973; George and Sherrington, 1984). Other workers have observed however, that some morphogenetic responses were not influenced by growth regulators only, but that growth regulators could interact with media components such as sucrose and nitrogen (Welander, 1976; Wetherall and Dougall, 1976; Jeannin et al., 1995; Ahn et al., 1996). The mechanism of auxin and cytokinin mediated morphogenesis has been the subject of extensive studies using different tissues and organs of various plant species (Murashige, 1974; Paranjothy, 1984; Lioseau et al., 1995). In addition other factors such as explants source and culture conditions affect the performance of in vitro cultures.

This work was therefore aimed at investigating the effects of some of these conditions on *Phoenix dactylifera* leaf explants cultured in vitro.

MATERIALS AND METHODS

Plant Materials

Offshoots of 2-3 years were harvested from mother palms and used as sources of explants for culture initiation. The different explants used were shoot tips, leaf bases and immature leaves.

Media

The basal medium for callus induction was that of Murashige and Skoog (1962) supplemented with 3% sucrose, 0.85% agar and 0.01-0.5% inositol. In addition, the following were also added: 0.002% aneurine hydrochloride and 0.3% activated charcoal. Different concentrations of NAA were initially tried for callus induction. From the preliminary findings, 100 mg/L NAA, or 100 mg/L 2,4-D and 3 mg/L 2-ip (Eke et al., 2005) was adopted for further work. This constituted the establishment medium. The pH of the medium was adjusted to 5.8. Explants were always incubated in the dark for callus induction while subcultures were done monthly.

Somatic embryo induction medium contained 0.05 mg/L NAA and 1 mg/L 2-ip while GA_3 was added at 2 mg/L for shoot elongation, retaining the same basal medium. Somatic embryos and shoots were cultured in 14 hours of light and10 hours of dark daily cycles. For root development, the MS basal medium used was supplemented with 30 g/L sucrose, 0.2 g/L glutamine and 0.15% activated charcoal as well as 0.05-0.1 mg/L NAA.

RESULTS

Callus could be initiated with a wide range of auxin concentrations although the time taken for both initial response and quantities produced varied significantly. From the initial trials on the effect of different auxin concentrations on callus formation, it was found that callus initiation and growth were best stimulated by a relatively high auxin concentration, 100 mg/L NAA or 2,4-D (Fig. 1). Further experiments were carried out in which the media were supplemented with NAA and one cytokinin at a time (Kinetin or 2-ip or BAP). The addition of cytokinin to the culture medium containing near optimal levels of NAA did not promote further noticeable growth at least in terms of quantity. It is conceivable however, that the addition of the cytokinin programmed the callus to cellular reorganisation thus opening the way for successful somatic embryo development. Date palm callus initiation is thus primarily under auxin control.

P. dactylifera callus could be induced at different sucrose concentrations (Fig. 2).

Callus fresh weight increased with sucrose concentration up to 0.1 M sucrose and then declined. The optimum sucrose concentration was found to be 30 g/L (Fig. 2) which is in agreement with Tisserat (1982). On the other hand, while nitrogen in the form of nitrate alone supported moderate callus formation and growth, ammonium alone was not very favourable for callus formation (Table 1). Both sources were needed by the explants for formation of appreciable quantities of callus. Synergy between the two sources of nitrogen is thus demonstrated. Sheat et al. (1959) reported similar effects explaining that NH_4^+ nitrogen can only serve as the sole source of nitrogen in a medium at a pH close to neutrality whereas the media used in this work had pH 5.8.

All three explants sources, meristems, leaf bases and immature leaves produced callus. In order of callogenesis potential, meristems were found to more readily form callus than leaf bases which in turn more readily formed callus than immature leaves. However, the ability to regenerate somatic embryos was much more enhanced in meristem derived callus when compared with the other two. Often, leaf derived callus was blocked at the callus stage and did not develop further. In general, callus could be obtained readily and reproducibly, usually at about the third subculture, from initiation. With more subcultures, the size of callus (Fig. 3a) grew. Initially, the callus appeared watery but the form of the callus became compact and globular over time with more subcultures.

It was possible to induce somatic embryo formation from the callus by using different media. Somatic embryos emerged reproducibly, reliably and uniformly on a medium that was supplemented with 0.05 mg/L NAA and 1 mg/L 2-ip (Al-Baiz et al., 2000). On the other hand, while hormone free medium also produced somatic embryos, this production was erratic. The somatic embryo (Fig. 3b) production could be either in the light or in the dark but normally the cultures are transferred to light once somatic embryos production started. The somatic embryos also developed into shoots in this medium. A number of shoots could grow up together which would be subsequently separated. The generation of multiple shoots (Fig. 3c) on agar solidified media have been recorded by other workers including Tisserat (1982) and Al-Baiz et al. (2000). As soon as the shoots were formed, they were transferred to a medium which contained 2 mg/L GA_3 to produce well formed shoots. This process took place in the presence of light (14 hours light and 10 hours dark cycles). Light intensity was initially low but was gradually increased as the somatic embryos began to develop into shoots. Transfer of the shoots to a medium supplemented with 0.05-0.1 mg/L NAA promoted root formation. Although roots could be formed in media with 30 g/L sucrose, much better roots were formed in media with higher sucrose levels of between 45-90 g/L. Optimum sucrose concentration for root formation with charcoal was 50 g/L. In vitro plants which brought forth roots could be acclimatized and transferred to soil. Some plants are currently in the nursery while some have been planted in the field undergoing observation.

DISCUSSION

In vitro multiplication is quite useful for date palm multiplication because of the dioecious nature of the palm which puts limitations on seed propagation for the production of planting materials. Different methods have been used by different workers many of which have been successful in many countries although refinements are in many cases still being made.

The date palm industry in Nigeria is relatively small but could potentially become very large. Nigeria has a population of over 140 million and has a considerable amount of trade especially in commodities, both within its borders and with the neighbouring countries. Already there is a lot of trade in dried date fruits locally within the country although most of the fruits are imported from North Africa and the Middle East. Consequently, there is huge scope for developing the industry in terms of production and production chain development. This will lead to a significant increase in secondary activities such as development and trade in inputs (planting materials, fertilizers, processing and related equipment, etc.).

We have successfully used meristems, leaves and inflorescences as sources of explants for date palm somatic embryogenesis. Leaves have the advantage of allowing a plant to be sampled without being itself destroyed. This leaf sampling technique is also sometimes applied in the oil palm and coconut (Duval et al., 1988; Verdeil et al., 1992). However, callus production is faster from shoot apices than from leaf explants. The combination of the growing shoot tip and the leaves as sources of explants, if so desired, could significantly multiply the harvest of callus and subsequently of plantlets from the mother plant.

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Table

Table 1. Effect of varying concentrations of organic and inorganic nitrogen on callus initiation and growth measured as callus mean weight (mg).

KNO_3 (mM)	NH_4 (mM)			
	0	10	20	40
0	0	0	0	0
10	0	220±5.3	180±2.6	0
20	316±4.5	570±6.1	145±2.8	50±0.3
40	250±3.6	920±10.3	90±1.1	20±0
60	200±3.6	720±9.1	60±1.0	20±0
80	170±2.6	200±3.1	0	10±0

Figures

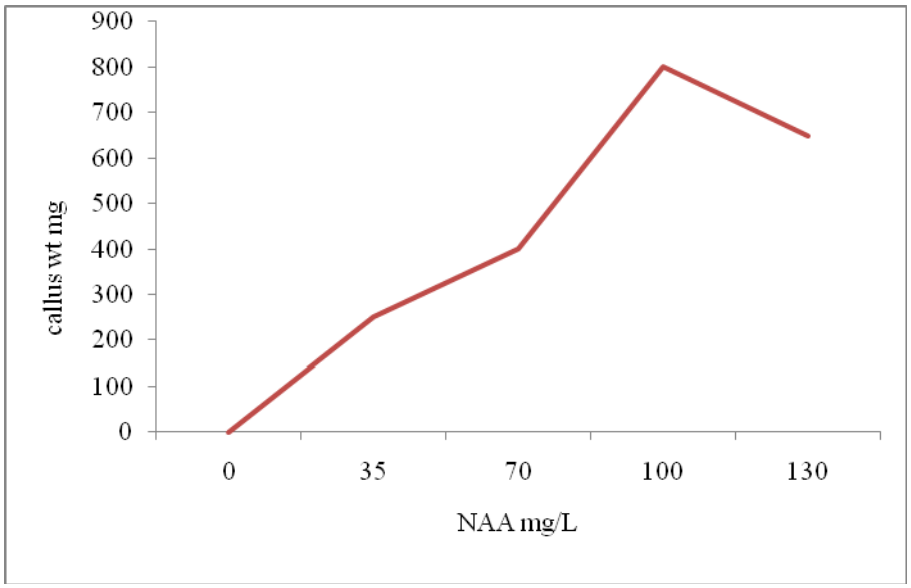

Fig. 1. Effect of combinations of NAA with BAP on date palm explants morphogenesis measured as mean fresh weight of callus produced.

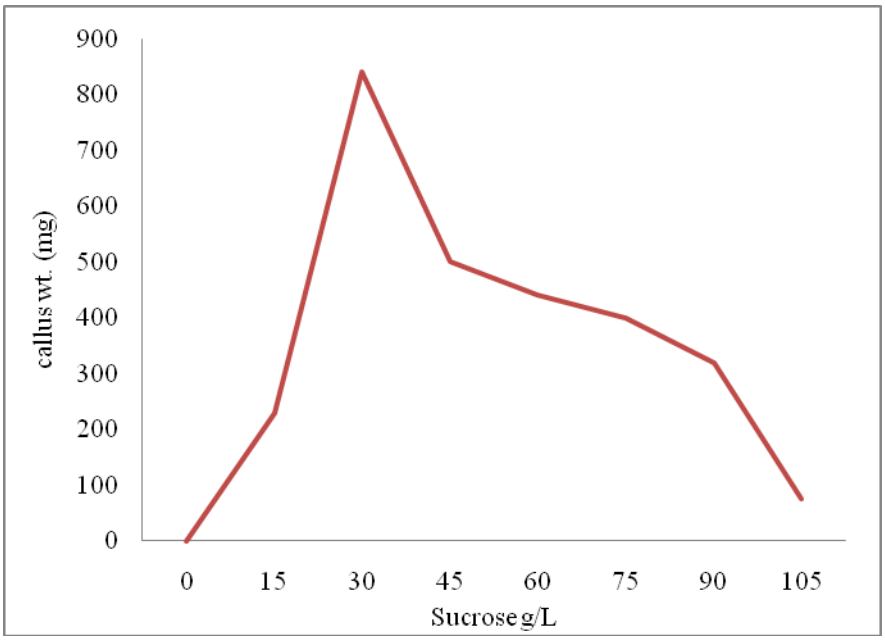

Fig. 2. Effect of sucrose and NAA concentration on callus induction measured as mean weight (mg) of callus obtained per treatment.

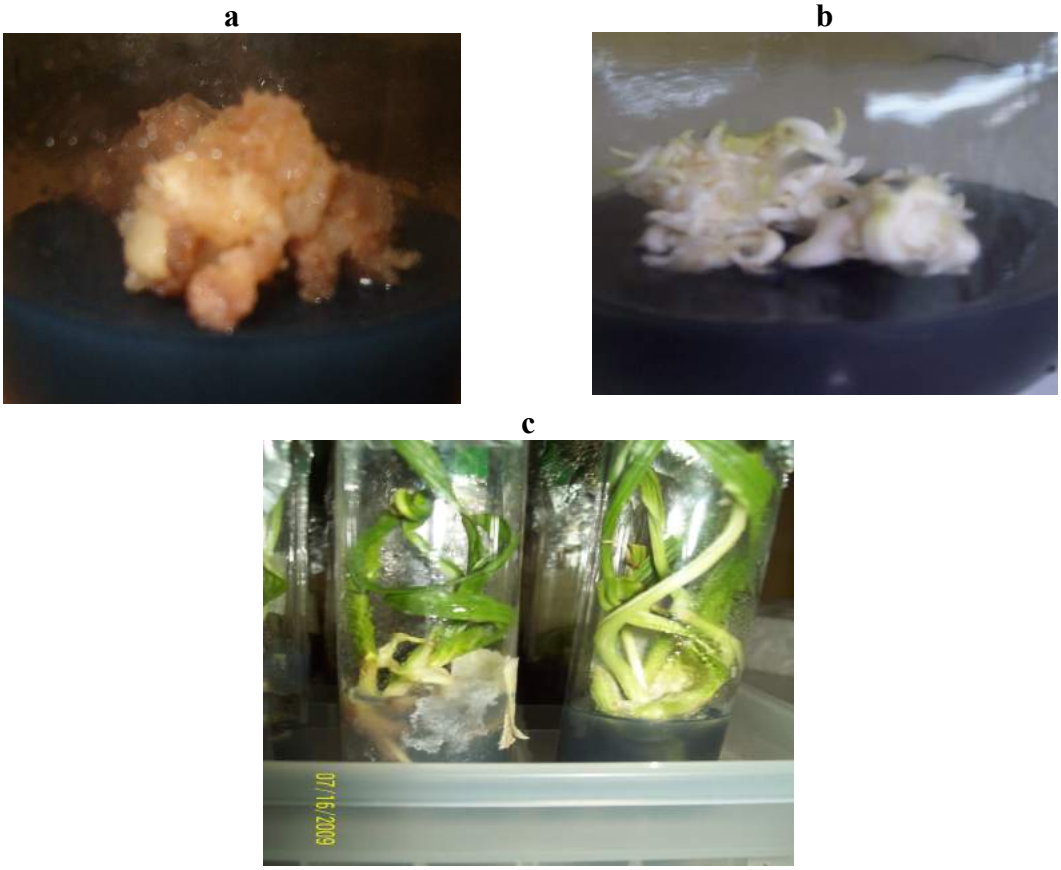

Fig. 3. Date palm development in culture (a) callus, (b) somatic embryos (c) shoots.

Evaluation of the Growth of Date Palm Seedlings Irrigated with Saline Water in the Sultanate of Oman

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Keywords: salinity, 'Khalas', 'Khunaizy', 'Abunarenjeh', 50% growth, Cl

Abstract

The Sultanate of Oman is an arid country (temperature ranges 7 to 50°C) located in the southeast corner of the Arabian Peninsula having a coastal line of almost 1700 km, bordering three seas: the Arabian Gulf, the Sea of Oman and the Arabian Sea. Rainfall (scarce and random) ranges between 50 to 300 mm with an annual mean of less than 100 mm. Irrigation water is highly scarce and groundwater (major part saline) is the main resource which ultimately causes soil salinization resulting in growth and yield losses of crops and desertification. Date palm is the major crop. Abandoning of date palm orchards has increased due to very low yields because of soil and water salinity. The situation required a systematic research. Therefore, this experimental work was conducted to investigate the water salinity tolerance of important date palm varieties. The study was performed to screen tissue derived date palm varieties for tolerance to water salinity [Treatments: A. Categories of irrigation water: EC_{iw} 3 (Control), 6, 9, 12, 15 and 18 dS m^{-1} B. Date palm varieties: 'Khalas', 'Khunaizy' and 'Abunarenjeh']. A uniform dose of N=0.375, P=0.20 and K=0.30 kg $plant^{-1}$ $year^{-1}$ was applied. Growth parameters: plant height, plant girth, number of new fronds and length of fronds were recorded annually for two years coupled with soil and plant analysis. The research work was undertaken at the Agricultural Research Center (ARC) Rumais, Oman during the years 2006-08 (split plot design with four replications in a low EC sandy loam soil having very good drainage). Fresh water (EC 1.0 dS m^{-1}) and the saline water (EC 35-40 dS m^{-1}) were mixed in appropriate ratios to get the desired levels of EC_{iw}. Data indicated a significant decrease in growth of all the three date palm varieties during irrigation for two consistent years with saline water of 6-18 dS m^{-1} when compared with 3 dS m^{-1}. However, a 50% decline was recorded only at water EC 18 dS m^{-1}. Therefore, date palm could be regarded as highly salt tolerant. Much difference between salt tolerance potential of the three varieties was not recorded, only 'Khunaizy' showed a little edge over the other two. A significant increase in leaf Na and decrease in K was observed while Cl remained unchanged. The physiological basis of salt tolerance in date palm was found as a strict control on Na and Cl concentration in leaves and keeping up the K content. It can be recommended that Date palm plants (seedlings of varieties 'Khalas', 'Khunaizy' and 'Abunarinjah') can be irrigated with saline water during vegetative growth. However, a significant decline in growth will be expected when the EC of irrigation water exceeds 9 dS m^{-1} that may reach up to 50% with water EC 18 dS m^{-1} (in sandy soil with very good drainage). The research has to be continued on irrigation with saline water at later stages of growth; the reproductive, fruit setting and maturity stages.

INTRODUCTION

The Sultanate of Oman is located in the southeast corner of the Arabian Peninsula.

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The country has a coastal line of almost 1700 km. The climate differs from one region to another. It is hot and humid during summer in the coastal areas and hot and dry in the interior regions while it is moderate in the winter. It is an arid country where annual rainfall ranges between 50 mm to 300 mm with an annual mean of less than 100 mm.

Groundwater is the main water resource of the country. The net annual natural recharge to the groundwater has been estimated to be around 1260 million cubic meters (MCM). The total water demand is around 1650 MCM of which 90% is used for agriculture. The deficit of 390 MCM is drawn from the groundwater reserves (Abdel-Rahman and Abdel-Majid, 1993). The cultivable area has been estimated at 2.2 million ha, which is 7% of the total area of the country, but is not less keeping in view the population. Over half of the agricultural area is located in the Batinah Plain in the north that has a total area representing about 3% of the area of the country. In Batinah, the Mean Annual Temperature is 28.6°C, relative humidity is 58%. The Batinah Plain is comprised of very thick alluvial, marine and aeolian sediments (Ministry of Agriculture and Fisheries, 1993).

During the last 30 years, there has been tremendous growth in agriculture. Excessive usage of groundwater resulted in seawater intrusion into the coastal aquifers and has caused the problem of soil salinity in many parts of the Batinah plain. The land resources are being curtailed rapidly due to soil salinity. According to a study of the Ministry of Agriculture and Fisheries (1993), 50% of the agricultural area in the South Batinah is affected from slight to moderate salinity (EC_e of >4 dS m^{-1}). Date palm is the overwhelming crop of the country.

Salt affected soils in Oman belong to only two orders; the aridisols and entisols and four suborders; salids, psamments, fluvents and orthents (Hussain et al., 2006). Such soils are conducive to reclamation in terms of structural stability. In North Batinah, about 50% of the total cultivated land is irrigated with water of salinity of above 3 dS m^{-1} and approximately 38% is irrigated with water of >5 dS m^{-1} salinity (Ministry of Agriculture and Fisheries, 1997).

The fresh water resources available for agriculture are declining quantitatively and qualitatively. Several countries have adopted the use of marginal water for irrigation to overcome water scarcity (Oron et al., 2002). This type of salinity caused by man's activities is called secondary salinity. Soil and water salinity has emerged now as a big problem of the present world's agriculture. The area of land affected by secondary salinity is steadily increasing, with recent worldwide estimates that indicated over 70 million ha of agricultural land as salt affected (FAO, 2005). Water salinity is one of the biggest problems of agriculture in Oman as well. It is more than 3.0 dS m^{-1} at many of the agricultural farms and may even reach 40.0 dS m^{-1} at certain places in the coastal belt of the Batinah region, the main seat of agriculture in the country. The categorization of groundwater was found to be 53, 18, 15, 6, 4, 3 and 1% that were having EC of 2, 2-3, 3-5, 5-7, 7-10, 10-15 and more than 15 dS m^{-1}, respectively (Hussain, 2005). In integrated studies of Batinah (1993-97) it was estimated that annual losses occurring from soil salinity to the country range from 7.311 and 13.966 million Omani Rial (OR). 'Khalas', 'Khunaizy' and 'Abunarenjeh' and a few others are the new popular varieties in Oman. Date palm trees have been grown in Oman for centuries. According to estimates, there are about 7.8 million plants growing at present. The area of date palm plantations has been estimated as 97059 ha. Naturally, this plant has also high tolerance against salinity. Nevertheless, at very high EC of water the plant can be affected negatively. The plants may totally dry out when irrigated continuously with very saline water otherwise their yields may decrease significantly to bring them below the economic level. The date palm production has been recorded as 0.28, 0.30, 0.24 and 0.20 million tons for the years 2000, 2001, 2002 and 2003 respectively while the mean yield/ tree was recorded as 280, 265, 219 and 201 kg for the same years indicating quite an unhappy situation (Ministry of Agriculture and Fisheries, 2004 update). Many big and small date palm farms have been abandoned now. A lot of variation in salt tolerance potential among different varieties is expected as well. However, noone knows exactly the salt tolerance limits of the varieties

presently growing or being planted newly in the Sultanate of Oman. The causes of low yield or abandoning of date palm orchards are as below.

Soil salinization due to continuous use of saline water and consequent low yields coupled with resultant desertification has become a big challenge and prime priority for the agricultural researchers of the country. However, no research work on these aspects has been conducted in the country so far. The exact salt tolerance limits of the presently growing major varieties as well as those being newly planted are to be investigated. Hence the objectives of this study were:

1. Evaluation of the water salinity tolerance levels of important date palm varieties of Oman.
2. Formulation of recommendations for the majority of the farming community who are growing date palm trees and vastly using saline water for irrigation of this most important crop of Oman.

MATERIALS AND METHODS

The growth rate of tissue derived date palm of different varieties under various irrigation water salinity levels was studied in these experiments for two years. The research work was undertaken at the Agricultural Research Center (ARC) Rumais (latitude 23°68'N, and longitude 58°01'E), Sultanate of Oman during the years 2006-07. Subsequent soil and plant analyses were completed in 2008. Homogeneous 16-months-old seedlings were transplanted in a permanent field in a split plot design.

Treatments

A) Categories of irrigation water: EC_{iw} 3 (Control), 6, 9, 12, 15 and 18 dS m^{-1}

B) Date palm varieties; 'Khalas Adhahirah' ('Khalas'), 'Khunaizy' and 'Abunarenjeh'. The statistical design was a split plot with four replications. Total number of plants was 72 (6×3×4=72). A non-saline soil was selected, leveled and prepared for transplantation of seedlings (16 months from germination stage) obtained from a tissue culture laboratory in the interior of Oman. Soil samples were collected just before transplantation and twice subsequently with one year interval. These samples were analyzed for EC and pH. The required levels of EC of water (EC_{iw}) were synthesized through mixing of fresh water (EC <1 dS m^{-1}) and the saline water (EC 35-40 dS m^{-1}). Plants were maintained through proper irrigation, weeding and protection. The growth data of height, trunk girth, number of new fronds (leaves), length of fronds, and leaf analysis of K, Na, and Cl elements were recorded annually.

Detailed Methodology

Separate supply lines of good quality (EC 1.0 dS m^{-1}) and ground saline water (EC 35.0-45.0 dS m^{-1}) were used for synthesizing the proper salty water according to the experimental treatments. A special irrigation system comprised of tanks, pumps, distribution pipes and drippers was set up. A uniform quantity of water was applied to all the plants in the field. The total quantity of water applied to each plant depended upon age and season, as proposed by Alnadi (2001) for date palm and different crops under Oman conditions. For the first year it was 3218.391 L (8.82 L day^{-1}). The amount of irrigation for the second year was increased by 50%, 4827.59 L (13.23 L day^{-1}). Organic matter was applied at the rate of 10 kg $plant^{-1}$ twice in two years during the month of February to all the plants. A uniform dose of fertilizer (NPK at the rate of 0.375-0.20-0.30 kg/ha) was applied to all the plants. Growth data were recorded annually (twice during the study) and each plot was analyzed for soil EC, pH and the following data were recorded. Data for the first and second year of the study have been presented separately. All the recorded data were processed statistically.

Irrigation and Experimental Soil

A coarse textured soil with good drainage was selected for the study because it represented the major soils of the country of Oman. It was a low EC soil (Table 3) with

alkaline pH and free from sodicity and with fertility. Soil samples were collected up to 60 cm at the end of the first growth year and up to 90 cm at the end of the second growth year in all the experiments. These samples were air dried and passed through a 2-mm sieve. Analytical methods of the US Salinity Laboratory Staff (1954) were followed unless otherwise mentioned. All the calculations were made on oven dried soil weight basis. The soil was analyzed for EC, pH, total nitrogen, potassium, available phosphorus, calcium, magnesium, sodium, carbonates, bicarbonates, chlorides, sulphates, calcium carbonates ($CaCO_3$), organic carbon and organic matter and also particle size analysis.

Plant Analysis

For plant analysis, 25 leaflets were collected from the bottom of each third frond from most recently fully developed five fronds (leaves) of each palm/sucker. Leaf samples were obtained twice after an interval of one year for determination of chemical parameters. Plant samples were ground to pass through 35 mesh after mixing of plant material obtained separately from each treatment. The ground material was preserved dry for subsequent plant analysis.

Sample Preparation and Analysis

An amount of 0.20 g was digested using conc. H_2SO_4 and was titrated through a Kjeldahl system for determination of N. An amount of 0.5 g of dried ground plant leaves of each sample was weighed into 100 ml Kjeldihal's tubes. A mixture of three acids; 60% $HClO_4$, HNO_3 and H_2SO_4 were added to each tube and was digested. The digest was used for the determinations of P, K and Na (Stewart et al., 1974; Method 12-5). Potassium and sodium were determined from the solution of the plant extract through the flame photometer and P was read through a spectrophotometer. For Cl an amount of 0.5 g was digested in a hot bath. The digest was titrated using silver nitrate.

Statistical Analysis

All the data collected were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980). Individual comparisons between treatments were made using LSD (Least Significant Difference) test. Some correlation coefficients (R^2) were also worked out.

RESULTS AND DISCUSSION

The systems created by nature are perfect. If the salt affected soils were emerging, there was also an evolution of salt tolerant plants able to tolerate such conditions. There is natural biodiversity in plants regarding their salt tolerance potential. According to Qureshi et al. (1998) there is a broad grouping of plants with respect to salt tolerance as halophytes, salt sensitive non-halophytes, and salt tolerant non-halophytes.

The tolerance against salts does not only vary from species to species but also within varieties as well as the growth stage (germination/seedling, vegetative and reproductive). Three varieties of date palm; 'Khalas', 'Abunarenjeh' and 'Khunaizy', famous in Oman, were therefore, included in this study. The growth performance of each variety under increasing levels of water salinity (3 to 18 dS m^{-1}) is as below.

RESULTS

Plant Growth Parameters

Overall, increase in plant height was gradually, but significantly retarded with all water salinity levels (EC_{iw} 6, 9, 12, 15 and 18 dS m^{-1}) when compared with the control (EC_{iw} 3 dS m^{-1}) whereas each consecutive level remained similar statistically in the first year (Fig. 1). At the end of the second year of study, overall deviations still extended and each water salinity level became different to the subsequent one in the increasing order of water salinity quantum. Thus, the impact of water salinity became more pronounced. 'Khalas' was observed to be taller than 'Abunarenjeh' while the latter was taller than 'Khunaizy'. The individual interactions of water salinity levels and varieties were also

significant in the second year and generally 'Khalas' was having higher values as compared to the other two varieties.

Figure 1: Gradual Effect of Water Salinity on Trunk Height of Date Palm Seedlings (n=4)

Eduction in bearing of new fronds by the date palm plants due to increasing water salinity levels was more pronounced in the second year of the investigation. The less bearing of fronds was found to be significant even in the first year with each increasing level (Fig. 2). The overall reduction of 8, 11, 21, 29 and 39% during the first year for water salinity levels of 6, 9, 12, 15 and 18 dS m^{-1} respectively increased to 12, 27, 32, 39 and 48% with relative percent values of 88, 73, 68 and 52 considering 100% for the control in the second year. The effect of each EC_{iw} level was found as significantly decreasing number of fronds. Therefore, overall reduction in number of fronds for each variety was also calculated for all the five water salinity levels that indicated curtailment values of 33, 31 and 30% in case of 'Khalas', 'Abunarenjeh' and 'Khunaizy', respectively.

Figure 2: Gradual Effect of Water Salinity on Number of Fronds of Date Palm (n=4)

The leaf growth may be reduced or totally stopped depending upon the magnitude of salinity stress. Water uptake by the roots may be decreased due to increase in osmotic pressure in the soil solution as result of salt concentration in the rhizosphere. The data of the present study also support this viewpoint. The average leaf length, when recorded at the end of the first year indicated a significant decrease by levels of water salinity beyond 6 dS m^{-1} (Fig. 3). The effect of 6 dS m^{-1} was not found to be significant in comparison to the control. The highest investigated level of 18 dS m^{-1} caused a maximum decrease in leaf length and differentiated statistically from all the other levels. The levels of 9, 12 and 15 dS m^{-1} were alike in their negative effect on leaf length during the first year. At the end of the second year, 'Khunaizy' was significantly higher than 'Abunarenjeh', but similar to 'Khalas'. In overall, water salinity proved negative and checked leaf growth significantly. For example, 3 dS m^{-1} was similar to 6 dS m^{-1}, but differentiated statistically from 9 dS m^{-1}.

Figure 3: Gradual Effect of Water Salinity on Leaf Length of Date Palm (n=4)

Under abnormal conditions, plants do not gain much girth and remain spindly and thin. Because this plant measure is differentiated relatively in longer time, therefore at the end of the first year the negative effect of water salinity levels of 6, 9 and 12 dS m^{-1} could not be assessed as significant compared with the control whereas levels of 15 and 18 dS m^{-1} still checked the thickening of plants and recorded girth was significantly lesser than the lower salinity levels (Fig. 4). These two levels were also statistically different. The variation behavior changed at the end of the second year of study and all the water salinity levels deepened in their negative effect. Each level was observed as having measurably lesser trunk girth when the quantity of salts in the water increased within the study. Even varietal differences became significant and overall trunk girth of 'Abunarenjeh' was lesser than 'Khalas' and 'Khunaizy', the latter two remained similar. However, interactions of EC_{iw} and different varieties could not be regarded as significant.

Figure 4: Gradual Effect of Water Salinity on Trunk Girth of Date Palm (n=4)

It was clear that in the second year varieties were significantly different from each other in all the four growth factors. Interactions between varieties and salinity after two years of growth were significant for only trunk height and number of fronds. 'Khalas' for these two parameters seems to be consistently the best. Also for the other two parameters it is almost the highest.

If overall growth pattern of date palm is examined, it may be regarded that EC_{iw} of 18 dS m^{-1} is the water salinity level that caused nearly 50% decrease of various plant parameters. The 50% reduction is considered the minimal economical value acceptable in salt tolerance studies because beyond that there is no benefit to grow plants under

consideration. At this level, the growth reduction will not be more than 50%. Of course losses will be less if water is having EC lower than 18 dS m^{-1} (Fig. 5).

Ionic Concentration in Date Palm Leaves

The concentration of Na in date palm leaves of different varieties increased with increasing level of water salinity when determined for the plants of one year old. Each consecutive level was similar to the lower or upper level but significantly different with alternative level. However, as the plants became relatively of more age (two years) they seemed to had become stronger and more salt tolerant. The sodium concentration in plant leaves did not differ statistically at this stage up to the level of EC_{iw} 9 dS m^{1} (Table 1). However, the level of 12 dS m^{-1} became statistically different from the control but remained similar to 6 and 9 dS m^{-1} as well as 15 dS m^{-1} on the upper side. The highest saline water (18 dS m^{-1}) caused a significantly higher concentration of Na in leaves as compared to the control and all the other water salinity levels (6, 12, and 15 dS m^{-1}). Differences between varieties remained non-significant in both years. Similarly, individual interactions of varieties and various water salinity levels were found to be insignificant.

Sodium could accumulate in cell walls of leaves or increase intercellular concentrations, leading to specific ion toxicity. High Na in leaf cells could easily cause loss of turgor (Ghafoor et al., 2004).

The role of K increases manifold when plants are growing in a saline environment. It is then used by the plants for osmoregulation and then it performs as an osmoticum. It neutralizes the negative effect of higher concentrations of Na in the tissues and controls its specific toxic effect. Salt tolerant plants exercise a preferred uptake of more K over Na through the ion selectivity phenomenon. The data of the present study also support this viewpoint. The effect of all the water salinity levels was observed as non-significant during the first year indicating the ion selectivity phenomenon, although Na concentrations enormously increased in the rhizosphere due to continuous irrigation with saline water.

However, at the end of the second year, the K concentrations in the date palm leaf tissue decreased significantly, in particular when the levels of water salinity were 15 and 18 dS m^{-1} (Table 1). The varietal differences after the first year were also significant. 'Khunaizy' and 'Abunarenjeh' had more K in their leaf tissues as compared to 'Khalas'. This may be the reason that 'Khalas' indicated relatively lesser salt tolerance than the other two varieties of date palm.

Generally, salt tolerant plants have higher limits of chloride toxicity. Some plants even check its entry into the root cortex and thus exercise ion selectivity. The data of the present experiment indicated that date palm has very strict check of Cl absorption from soil solution even though it may be highly saline. The non-significant effect on Cl concentration in date palm leaves was recorded in both years (Table 1). The differences between varieties as well as all interactions were also found insignificant. The correlation in between water salinity and leaf Cl content as well as soil salinity and leaf Cl concentration were computed as non-appreciable in statistical terms. This indicated that a strict control on Cl content within the plant is the mechanism of salt tolerance in date palm.

Soil and Water

The Soil EC_e increased significantly with each level of water salinity as compared with the control when either observed after one year or two years (Fig. 6). Nevertheless, EC_{iw} 6 dS m^{-1} was found to be similar with 9 dS m^{-1} while 12 dS m^{-1} was alike with 15 dS m^{-1} during the second year. Varietal differences regarding soil EC_e as well all interactions were evaluated as non-significant.

DISCUSSION

It was observed that deleterious effects of various water salinity levels (6, 9, 12,

15, and 18 dS m^{-1}) on all these parameters increased with time because the salts being added through irrigation water kept concentrating gradually and increased soil EC significantly (Fig. 5). If the overall growth pattern of date palm is examined, it may be regarded that EC_{iw} of 18 dS m^{-1} is the water salinity level that caused nearly a 50% decrease of various plant parameters. The 50% reduction is considered the minimal economical value acceptable in salt tolerance studies because beyond that there is no benefit to grow plants under consideration. Hence, it may be concluded that when date palm is planted in coarse textured soils (sandy loam, loamy sand and sand that dominate in Oman) with very good drainage it can be irrigated with water of EC up to 18 dS m^{-1} during vegetative growth. There were more percentage/less reductions in the three growth characters out of four for 'Khalas' in comparison to the other two varieties. When 'Abunarenjeh' was compared with 'Khunaizy', each variety was found superior over the other in two characters. Hence, it may be concluded that 'Khunaizy'/'Khalas' was having a little edge over other two varieties as regards to salt tolerance potential.

Accumulation of salts in the root zone affects plant performance through creation of water deficit and disruption of ion homeostasis (Munns, 2002) which in turn causes metabolic dysfunctions. Irrigation water and the resultant secondary salinity arising from this field operation had a growth retarding effect on date palm plants. The mechanisms of this negative effect may be: osmotic effects that restrict water and nutrient uptake, specific ion effect of Na and Cl ions, nutritional imbalance and inhibition of physiological processes like photosynthesis, transpiration, energy reactions, metabolism of nutrients and use of extra energy in osmoregulation. Shani et al. (2001) related the yield loss in date palm to reduced photosynthesis, high energy and carbohydrate expenses in osmoregulation, and interference with cell functions under saline conditions while Ghadiri et al. (2005) reported restricted water uptake by salinity due to the high osmotic potential in the soil and high concentrations of specific ions that may cause physiological disorders in the plant tissues and reduce yields. It has been reported that environmental stresses, especially water stress and salinity, increase the generation of toxic active oxygen species (O_2^-, H_2O_2 and OH) that result in cellular injury or damage which frequently results from impaired or perturbed metabolism (Sairam and Saxena, 2000; Sreenivasulu et al., 2000). Salinity also induces changes in the plant hormonal balance and reduction of the levels of growth-activating hormones such as auxins and cytokinins (Glyan´Ko et al., 2005). Yousaf and Award (2007) observed a strong linear correlation between the chlorophyll a/b ratio and assimilation rate throughout salinity treatments. The slope and the correlation coefficient between the chlorophyll a/b ratio and assimilation suggested that salinity reduced assimilation predominantly via the reduction in chlorophyll a contents.

According to criteria of Mass (1986), the date-palm plant is included in the group that is tolerant to salinity with a threshold for reduction of yield at soil EC of 4.0 dS m^{-1} and has 50% reduction in yield at 17.9 dS m^{-1}. He observed good yield at EC_{iw} of ground water around 10.0 dS m^{-1}. Whereas Ayers and Westcot (1985) proposed the threshold of date palm as 4.0 and 2.7 dS m^{-1} for soil and water respectively and respective 50% yield reduction limits were found by them as 18.0 and 12.0 dS m^{-1}. However, the salt tolerance level can vary a lot depending upon texture and drainage of soil, climate and the genetic potential of the variety. Marcar et al. (1995) reported that date palm (*Phoenix dactylifera*), depending upon variety can tolerate up to at least 18000 ppm salts (28.1 dS m^{-1}) or even more. However, fruit production usually stops at about 10000 ppm (15.6 dS m^{-1}) salts. In a relatively recent greenhouse experiment of Al-Hammadi (2006) optimal growth was found in control and 3000 ppm (4.7 dS m^{-1}) of NaCl. The relative growth rate (RGR) and biomass decreased significantly by increasing salinity to 6000 (9.4 dS m^{-1}) and 12000 ppm (18.8 dS m^{-1}). Results of their study were closer to the present investigations because the soil was coarse with very good drainage, hence a higher water salinity level (18 dS m^{-1}) caused about 50% reduction in various growth parameters.

Sameni and Morshedi (2000) reported that saline-sodic irrigation water, coupled with the low annual rainfall and high evaporation and transpiration (common in the arid

and semi-arid regions) resulted in accumulation of soluble salts in the soil solution, which altered the structure and, consequently, affected the soil hydraulic conductivity. Net salt accumulation is highly dependent upon soil texture and drainage. If the soil is light in texture with good drainage, there will less seasonal salt accumulation in comparison to soil with fine texture and restricted drainage, although climatic conditions especially rainfall also play a very important role in leaching of salts.

Soil pH; the experimental soil was slightly alkaline with a pH of 7.7. The irrigation with saline water for two years indicated a significant increase in this important soil characteristic and made it more alkaline. The EC_{iw} 6 dS m^{-1} was assessed similar to the control (3 dS m^{-1}) on the lower side and alike 9 dS m^{-1} on the upper side. The water salinity level of 9 dS m^{-1} was statistically similar to 12 dS m^{-1} that in turn resembled 15 and 18 dS m^{-1}. All the water salinity levels were found to be significant in comparison to the control except 6 dS m^{-1}. Such a trend was noticed in both the years. Hence soil pH also increased accordingly most probably due to the increase in Na ion.

The resulting increase in soil EC retarded plant growth. This was mainly due to osmotic effects of accumulated salts that reduced water uptake of water and nutrients. An imbalanced ionic uptake also occurred that affected plant nutrition negatively, especially Munns (2002) found that accumulation of salts in the root zone affected plant performance through creation of water deficit and disruption of ion homeostasis which in turn caused metabolic dysfunctions. Paridaa et al. (2005) illustrated that salinity is the major environmental factor limiting plant growth and productivity. The detrimental effects of high salinity on plants were observed at the whole-plant level as well. The growth rate directly depends upon the products of various metabolic processes. Therefore, any disruption in normal metabolism will naturally be translated into curtailed growth and less yield.

Control on Chloride Uptake

The anions attached with Na are very important because negative effects of salinity on plants are also determined by them. The same quantity of Na when attached with chlorides prove more toxic than sulphates, but lesser than carbonates and bicarbonates. However, the quantities of the latter two are generally lower in the saline soil than the former two, but it was specifically true for the experimental soil. The irrigation water also contained very large quantities of chlorides, particularly when its EC was very high in last the two to three treatments. Hence, the detrimental effect of chlorides could greatly harm the date palm plants but surprisingly amounts determined in the plant leaves were very low and the treatment effect was found to be non-significant. The correlation of leaf chloride was also determined to be non-significant. Therefore, it can be concluded that date palm exercises a strict control on Cl uptake as a salt tolerance mechanism.

ACKNOWLEDGEMENTS

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Tables

Table 1. Effect of water salinity on leaf Na, Cl, and K % of date palm.

Water salinity levels EC (dS m^{-1})	Na %	Cl %	K %
3 (Control)	0.24 D	0.99 NS	1.74 A
6	0.25 CD	1.00	1.68 AB
9	0.26 CD	1.01	1.63 AB
12	0.27 BC	1.04	1.60 B
15	0.29 B	1.06	1.59 B
18	0.32 A	1.08	1.58 B

Values are mean of four replications.
Values sharing the same letters are non-significant at 5 % level of significance.

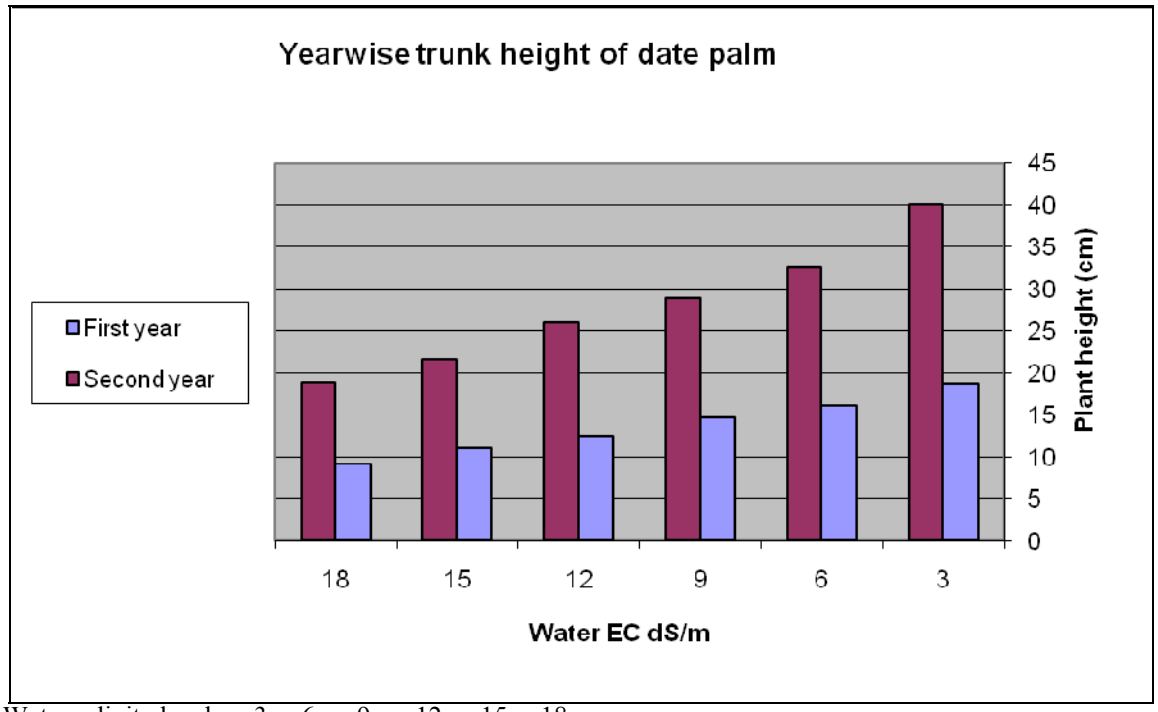
Table 2. Original soil analysis of three field experiments.

Sr. No	Determinations	Unit	Value
1	Saturation percentage	%	22.7
2	pH_s	-	7.7
3	EC_e	$dS.m^{-1}$	2.77
4	Na^{+1}	Meq L^{-1}	6.6
5	K^{+1}	Meq L^{-1}	0.5
6	SAR	(m mol L^{-1})$^{1/2}$	0.89
7	Total nitrogen	%	0.0033
8	Available phosphorus	$mgKg^{-1}$	12.4
9	Textural class	-	Loamy sand

Table 3. Analysis of irrigation water used in the experiments.

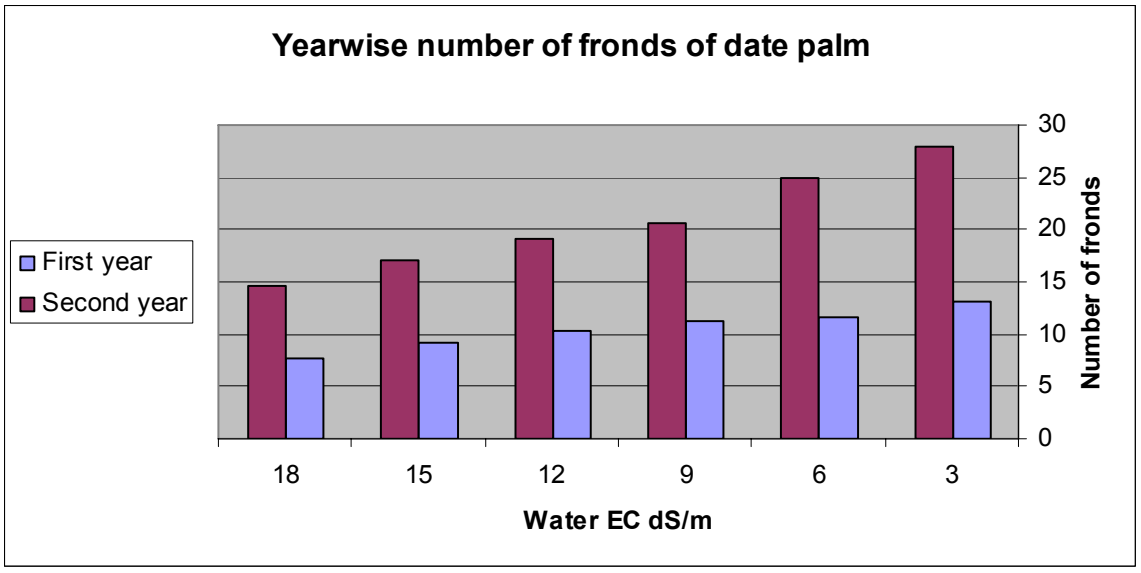
Sr. No.	Determinations	Units	EC 3 dS/m	EC 6 dS/m	EC 9 dS/m	EC 12 dS/m	EC 15 dS/m	EC 18 dS/m
1	Electrical Conductivity (EC)	dSm^{-1}	3.2	6.1	8.9	11.9	15.1	18.1
3	pH	-	6.2	6.5	6.7	6.9	7	7.4
4	Carbonates (CO_3^{-2})	$mmol_c\ L^{-1}$	Traces	Traces	Traces	Traces	Traces	Traces
5	Bicarbonates (HCO_3^{-1})	$mmol_c\ L^{-1}$	0.7	0.8	0.7	0.7	0.9	0.9
6	Chlorides (Cl^{-1})	$mmol_c\ L^{-1}$	28	56	83	113	150	180
7	Sulphates (SO_4^{-2})	$mmol_c\ L^{-1}$	10.4	8.5	5.3	12	13.5	16
8	Calcium	$mmol_c\ L^{-1}$	2.6	5.6	8.2	12.4	14.4	14
	Magnesium	$mmol_c\ L^{-1}$	10	21.8	34.2	46.8	59.2	76
9	Sodium (Na^{+1})	$mmol_c\ L^{-1}$	26.3	37.4	45.9	54.1	62.8	71.5
10	Sodium Adsorption Ratio (SAR)	$(mmol\ L^{-1})^{1/2}$	7.4	10.1	10.0	9.9	10.4	10.7
11	Residual Sodium Carbonate (RSC)	$mmol_c\ L^{-1}$	Nil	Nil	Nil	Nil	Nil	Nil

Figures



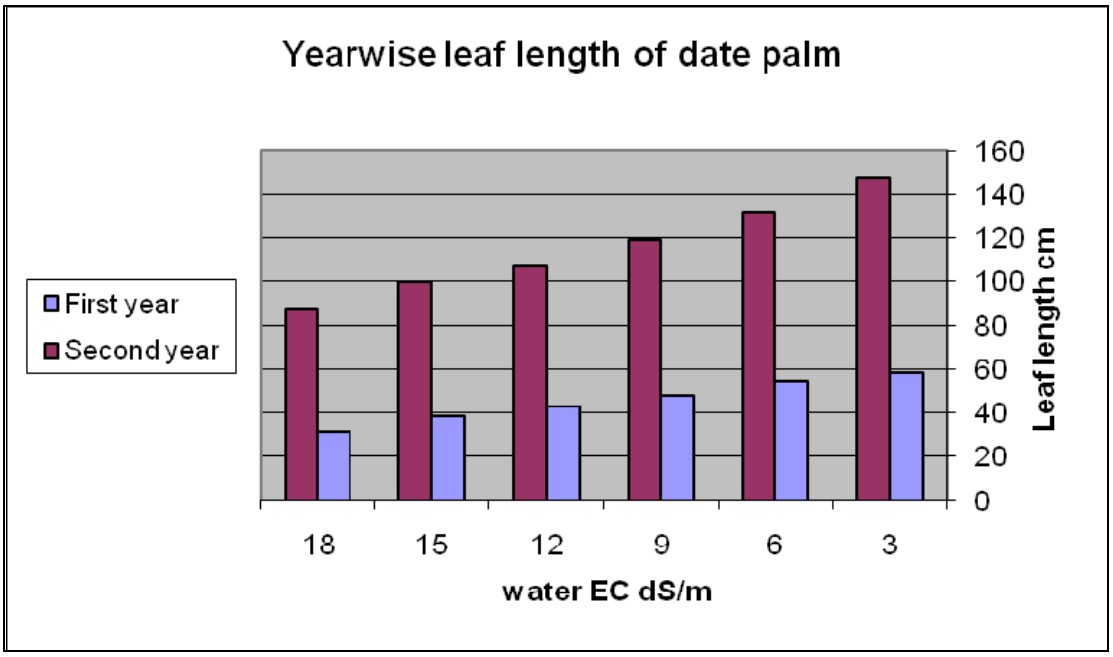
Water salinity levels	3	6	9	12	15	18
First year	A	B	BC	CD	DE	E
Second year	A	B	C	D	E	F

Fig. 1. Gradual effect of water salinity on trunk height of date palm seedlings (n=4).



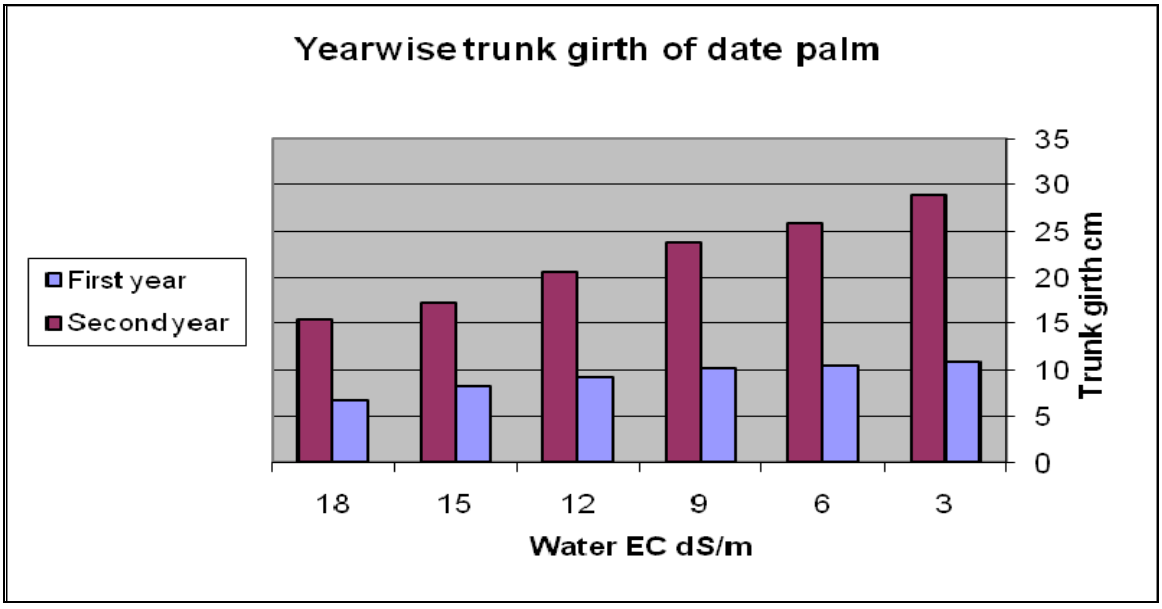
Water salinity levels	3	6	9	12	15	18
First year	A	B	B	C	E	E
Second year	A	B	C	CD	D	E

Fig. 2. Gradual effect of water salinity on number of fronds of date palm (n=4).



Water salinity levels	3	6	9	12	15	18
First year	A	A	B	BC	C	D
Second year	A	B	BC	CD	DE	E

Fig. 3. Gradual effect of water salinity on leaf length of date palm (n=4).



Water salinity levels	3	6	9	12	15	18
First year				NS		
Second year	A	B	C	D	E	F

Fig. 4. Gradual effect of water salinity on trunk girth of date palm (n= 4).

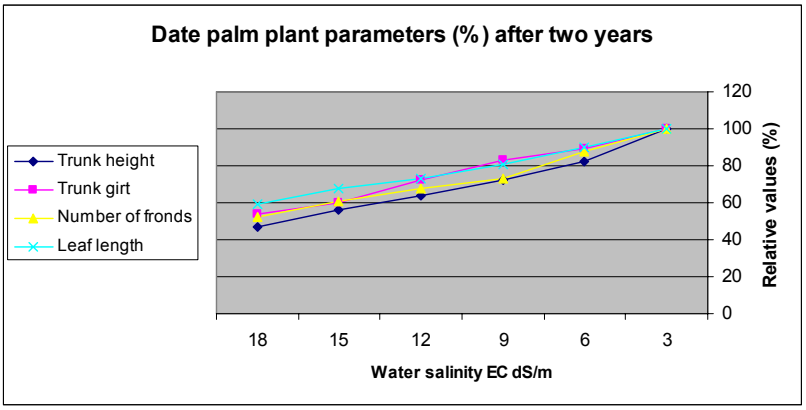
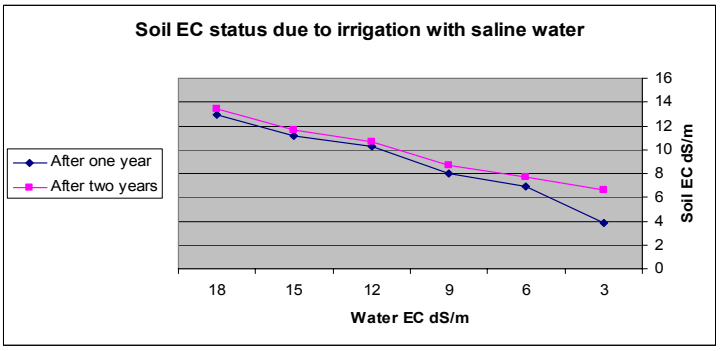
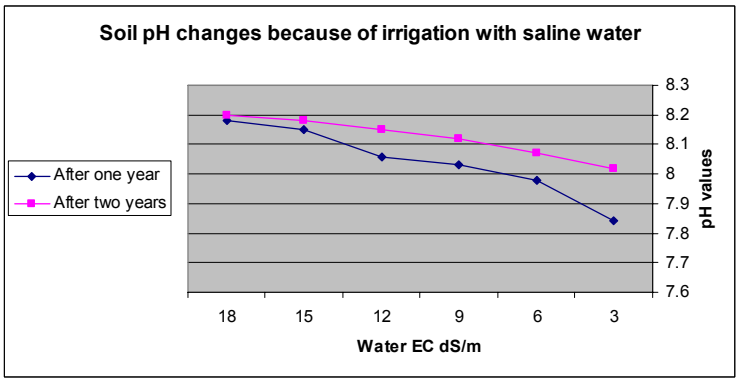


Fig. 5. Effect of water salinity on plant characters of date palm varieties.



Water salinity levels	3	6	9	12	15	18
Significancefirst year	E	D	C	B	B	A
Significance second year	D	C	C	B	B	A

Fig. 6. Effect of water salinity on soil EC after two years (n=4).



Water salinity levels	3	6	9	12	15	18
Significancefirst year	C	BC	AB	AB	A	A
Significance second year	D	CD	BC	AB	A	A

Fig. 7. Effect of water salinity on soil pH after two years (n=4).

Next Generation DNA Sequencing Applied to the Date Palm Tree (*Phoenix dactylifera*)

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Abstract

Date Palm, a dioecious monocot with historical records of cultivation of over 5000 years, is a fruit producing tree important in agriculture and tradition in the middle east and other arid regions of the world. The date palm's ability to withstand extremely harsh conditions, while producing highly nutritious fruit with relatively minimal care, makes it a good candidate for improving arid land agriculture. Despite the importance of date palm in the agriculture of many countries, relatively little is known about its genetics. To provide the foundation for date palm genetic studies the genome of a female date palm tree was sequenced using massively parallel shotgun sequencing by synthesis technology. A de novo assembly was generated using only the shotgun reads and was annotated for both genes and parental allelic differences. To assist future studies on possible genetic differences between male and female trees, we generated light shotgun sequencing of 3 male trees and 1 additional female tree. We report large scale polymorphisms between these genomes. With this information date palm researchers will now be able to begin genetic research in earnest as they have essentially the full set of date palm genes available to them. From this they will be able to more thoroughly understand how disease affects certain varieties over others, fruit differences, and a host of other genetically based differences in date palm. We have also provided a large set of novel DNA markers (SNPs) which will allow much more accurate typing of various date palm varieties. Researchers can access the genome with ease and use it in their research.

RESEARCH

The date palm genome is of extreme importance to the Middle East in tradition, religion, economy and nutrition among others. With a long history of cultivating the date palm, the Middle East has a responsibility to develop the date palm. One initial step in this process is understanding the genetic makeup of the date palm and also genetic diversity within the species. These two critical pieces of information would assist preservation and development of the date palm.

To this end we undertook both sequencing and comparative genomics of the date palm genome. DNA sequencing was conducted to provide and initial genome sequence and gene set for all future genetic studies. The comparative genomics was conducted to understand more of the genetic diversity of this important plant. To date most genome sequencing projects have been conducted with the classical 'Sanger Sequencing' technology. The Human Genome, mouse genome, and Arabidopsis genome (first plant genome) were all sequenced using this technology at a very high cost over a long period of time. Within the last 3 years DNA sequencing has seen a change in technology that has dropped both the time and cost of generating a genome sequence by over 1000-fold. These approaches, termed 'Next Generation DNA Sequencing' allow a massively parallel approach to obtaining a genome sequence. We attempted to use this technology for genome sequencing. Indeed we were the first group to report our results on a plant genome giving free access to the data generated in this fashion.

The date palm genome was predicted to be approximately 250,000,000 base pairs long but according to our sequence we estimate it to be between 550,000,000 and

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650,000,000 base pairs long. With such a large genome we needed to take a modified approach to genome sequencing to make it amenable to the 'Next Generation Sequencing' data which provides hundreds of millions of sequences, albeit very short in length (~50 bp). The details of the approach are depicted in Figure 1. The first step, assembly required that we join 234 million sequences ranging in size from 36 to 64 bp in length. This was accomplished using the VELVET software (Zerbino et al., 2008) and over 70 Gb of RAM. This 'Assembly' step joined the short reads into contiguous sequences of approximately 500 base pairs (bp). These were used as input for the next step, 'Paired End Assembly', where sequence information from either end of a DNA molecule was used to join the short pieces into even longer fragments termed 'microscaffolds'. Upon completion of this step we added long mate pair information in the 'scaffolding and validation' step to further increase the length of the contiguous stretches of DNA (termed Scaffolds). At this point we had reached an N50 size of 14,840 bp which is significantly longer than the typical date palm gene of 4,106 bp. This ensures that the genes are very likely to be full length. We proceeded to 'Annotation' where we detected over 19,000 genes and have added those to our website for the date palm community to use. Independent analysis of our genome assembly showed that over 90% of genes were predicted while over 74% of them were present in full-length. This suggests that the genome sequence provided to the community is a high-quality draft sequence from the perspective of gene finding. Furthermore, DNA sequencing of 5000 bp of the genome using the older Sanger Sequencing technology revealed that there was no mistake in the next-generation sequencing results. Any discrepancies with the sanger data proved to be correct polymorphisms detected later.

To better understand the genetic diversity within the date palm species we attempted to document genomic variation from the genome sequence. Using the original shotgun sequences we were able to predict single nucleotide polymorphisms (SNPs) between the parental alleles of the date palm. In this case we detected over 700,000 SNPs between the two parents as transferred to the daughter tree. This is assist in unambiguously genotyping date palms. We continued our work in understanding date palm genomic variation by light sequencing of 4 other date palm genomes - 3 male genomes and one additional female genome. Large scale difference between two genomes can be detected by comparing the counts of sequences from each genome which matches a reference genome (Fig. 1., 'CNV finding'). By using a sliding window of appropriate size, large differences in sequence number between the genomes indicates a possible copy number variation (CNV). CNV analysis of the 4 sequenced genomes revealed 1000's of CNVs of which over 7,000 overlapped a gene region by more than 50% of its length. This tremendous amount of variation in the genome size was surprising and offers a glimpse into the genetic diversity of date palm. It gives a start for understanding the differences between male and female trees.

CONCLUSIONS

We have provided the first genome sequence of the date palm genome and made it publicly available to all researchers. We hope this will be a catalyst for future date palm genetics research. Furthermore, we provided gene annotation and polymorphism information along with the genome sequence. The gene annotation should assist in starting to understand the unique traits of the date palm in its ability to thrive in certain environments. The polymorphism information will assist in developing polymorphic markers with which to map traits of interest and better genotype the present date palm population. At present there are only approximately 30 polymorphic markers used in date palm. We present here 700,0000 SNPs which could be used to design a genotyping array to unambiguously classify date palm cultivars. Previously this was only possible using things such as taste, smell, color, and a small set of markers. Large scale genomic variation in the form of CNVs was also reported and the tremendous amount of variation, even in gene regions, was a surprise though other plants do show large amounts of variation. We believe the CNV resource will allow other researchers to begin

understanding how much genetic variation there is within date palm and what approaches can be used to preserve genetic variation within the plant. This genetic variation is critical to the long term survival of date palm as a wide 'genetic bank' within the species provides it new genes to draw on in the case of abiotic or biotic challenges. We hope the research here will catalyze date palm genetic research and provide a foundation for researchers to begin long awaited studies.

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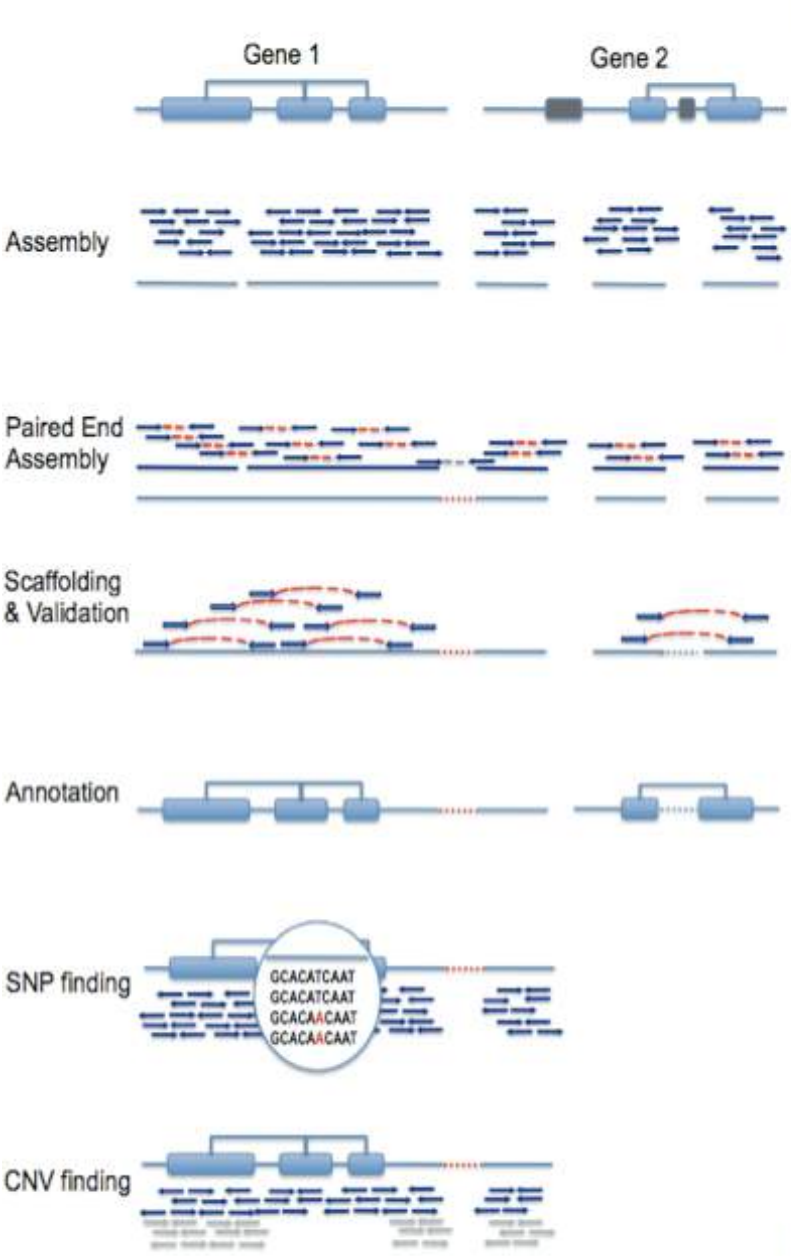
Figures



Date Palm Genome	
GC%	37.5%
Genome Size Estimate	550 Mbp
Assembly	
N50 Contig Lngth	504 bp
Total Contigs	1,318,993
Total Sequence Reads	234,205,452
Estimated Read Coverage	25X
Paired End Assembly	
N50 Microscaffold Lngth	4,243 bp
Total Contigs	145,298
% Full Length Genes	58%
Scaffolding	
N50 Scaffold Lngth	14,840 bp
Total Scaffolds (min 200bp)	73,747
% Full Length Genes	73%
% Genes Captured	91%
Total Scaff Lngth (non gap)	282 Mbp
Annotation	
Predicted Genes	19,414
Average Gene Size	4,106 bp
Genes with Blast Similarity	17,667
SNP Finding	
SNP Base Calling Coverage	15.4X
Total Number of SNPs	768,957
CNV Finding (4 genomes)	
Range of CNVs/genome	1,480-18,712
Total Genes in CNVs (any genome)	7,922

Fig. 1. Date palm genome sequencing process.

On the Status of Chromosomes of the Date Palm (*Phoenix dactylifera* L.)

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[3] Date Palm Expert, Date Palm Global Network

Abstract

The present article summarizes the status of the existing data on the issue of chromosome number and karyotype studies of the date palm, *Phoenix dactylifera* L. The literature on this subject is rather heterogeneous and confusing, and the number of chromosomes reported ranged from 2n=26 to 2n=36, in addition to the possible occurrence of polyploidy and aneuploidy. As early as 1910 Nemec examined young embryos of *P. dactylifera* and reported the presence of 2n=28 chromosomes. Beal (1937), however, observed 18 pairs of chromosomes (18 bivalents) as seen in M1 in the microsporocytes of 5 male cultivars of date palm; and 2n=36 chromosomes in root tips of 6 varieties of both male and female plants. Beal also observed in 2 other species of the genus *Phoenix* 1n=18 in *P. canariensis* and *P. sylvastris*; and 2n=36 chromosomes in 3 other species of the genus.

Selbi (1988) in his analysis of the chromosome karyotypes of both male and female date palms, indicated an identical chromosome number for both; and he reported 2n=36. Loutfi (1989) in his study on tissue culture-derived date palm plants obtained from inflorescence culture, found that the plants produced were morphologically identical to plants produced via organogenesis, and the chromosome number for these plants were strictly diploid. Ibrahim et al. (1998) also reported 2n=36 in some cultivars of date palm. Surprisingly, however, a chromosome number of 2n=26 was observed in two Moroccan date palm cultivars in the in vitro generated plantlets (Loutfi, 1999). The establishment of a date palm genomic map is important; and cytogenetic studies to firmly establish the chromosome number, and to detect chromosomal aberrations; expansion of date palm Gene Bank and biodiversity management using molecular tools, also represent important goals. The application of in situ hybridization, fluorescent in situ hybridization (FISH), spectral karyotyping (SKY) and other techniques to identify the chromosomes and karyotype, and to detect chromosomal aberrations and clonal variations; and early detection of gender in seedlings resulting from seed germination, should also not be ruled out. Although Sijak-Yakovlov et al. (1996) have detected the presence of sex chromosomes carrying distinctive nucleolar heterochromatins, based on chromomycin staining, nevertheless, a rather simpler tool for early detection of gender in date palm is of great importance. As early as the 1980's we have recognized an early disjunction of one pair of the 18 bivalents in some male cultivars of date palm during microsporogenesis at early A1 of the first meiotic division (Al-Ani and Nawal Abdullah, 1987); and through personal communication (Al-Mayiah et al., 2009) counted 2n=36 in some date palm cultivars, and detected the presence of sex chromosomes. Furthermore, they have detected some polyploidy and aneuploidy during their cytological investigation. Finally, the date palm whose seeds stand tolerant and totipotent; and maintained its true-to-typeness over a period of more than 2000 years, deserves more effort from biologists and other scientists to solve some cytogenetical problems associated with this socio-economically important crop, the 'blessed tree'. It is suggested that some of these investigations may be a partial fulfillment of getting a higher degree at UAEU.

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INTRODUCTION

In spite of the progress achieved in agricultural, commercial, industrial, micropropagation, as well as many aspects of date palm fruit researches, there has been but a limited progress in genetical and cytogenetic aspects of this socio-economically important crop.

The family *Palmae*, to which the genus *Phoenix* belongs, contains more than 200 genera and 2,280 species. Chromosomal data are still relatively scarce with a little more than 400 counts having been published up to the mid 1980's (Johnson, 1985), though some progress has been achieved later (Johnson and Brandham, 1997). The relatively few chromosome counts made in members of the family *Palmae* had been attributed to the difficulty in obtaining rapidly growing root tips in mature trees, suitable for chromosomal examination. Palms tend to be slow-growing throughout the year, thus producing very limited root systems. Furthermore, the chromosomes are small and numerous, and thus do not lend themselves to cytogenetic investigations (Johnson 1985; Johnson and Bradham, 1997).

In eukaryotes the diploid chromosome number ranges from 2n=2 as in *Haplopappus* (a desert relative of sunflower) and in the fungus *Penicillium* to 2n=1,262 in the Adder's tongue fern (Johnson and Raven, 2001).

As early as 1910, Nemec examined young embryos of *P. dactylifera* and reported the presence of 2n=2×=28 chromosomes in a non-specified cultivar of date palm.

However, since the early work of Beal (1937) to date, most cytologists reported 1n=1×=18 and 2n=2×=36 as haploid and diploid numbers of chromosomes, respectively, in several male and female cultivars of date palm.

The diploid chromosome numbers have been studied in root tips of six species of the genus *Phoenix* and in six varieties of *P. dactylifera*. In these studies, Beal recognized the same diploid number of chromosomes (2n=2×=36) and the same general range of size and form variations, as seen in mitotic metaphase. Especially worthy noting is the marked similarity shown in the somatic chromosome complements of the six varieties of *P. dactylifera* studied. However, the diploid chromosome numbers (2n) in Beal's investigations were obtained from mitoses in root tips of young seedlings, obtained from seeds belonging to the studied species or cultivars, and not from root systems of old trees.

In addition to *P. dactylifera*, the 5 other species studied by Beal were; *P. canariensis*, *P. honceana* var. *formosanum*, *P. humilis*, *P. reclinata* and *P. sylvestris* (Table 1). The haploid chromosome number was obtained from staminate flowers born on male palms. Microsporocytes in metaphase of the first meiotic division (M1), were used to determine the haploid number of chromosomes, by counting the number of bivalents at M1. Microsporocytes at M1 of *P. dactylifera*, *P.canariensis*, *P. sylvestris* showed 18 bivalents, and the complements in each revealed a marked similarity in the range of size and form variations among bivalents of those species. Both *P. canariensis* and *P. sylvestris* have been successfully used as pollen parents in crosses with *P. dactylifera*. Although the chromosomes of the former two species of *Phoenix* tend to be somewhat smaller compared with those of *P. dactylifera*, nevertheless, Beal has concluded that this seems not to be of special significance as they breed truly with *P. dactylifera.*

Few other cytologists, however, reported different chromosome numbers, ranging from 2n=2×=18 to 2n=2×=36 as a diploid chromosome number in several male and female cultivars of date palm. Some of the most important references dealing with chromosome counts have been compiled in Table 1.

Al Salih and Al Rawi (1987) and Al Salih et al. (1987) reported 2n=2n=32 or 2n=2×=36 in two female cultivars of date palm. However, they encountered variation in chromosome number such as 28, 32, 34, 36 and 64.

Selbi (1988) in his analysis of chromosome karyotypes of male- and female date palms, indicated identical number of chromosomes for both, and reported 2n=2×=36. This investigation was conducted to deny the hypothesis raised at a time by some workers, which postulates that female date palms may be a tetraploid (4n) and that the process of pollination merely stimulates the development of the embryo from a diploid egg without

fertilization. However, this hypothesis was rejected by some cytologists who determined that both male and female palms are strictly and consistently diploids (2n).

Lufti (1989) in his study on tissue-culture derived date palm plants obtained from inflorescence culture, found that the plants produced were morphologically identical to plants produced via organogenesis, and the chromosome numbers for these plants were strictly diploid, with 2n=2×=36. Ibrahim et al. (1998) also reported 2n=2×=36 in some cultivars that he studied.

Surprisingly, however, a chromosome number 2n=2×=26 was observed in 2 Moroccan date palm cultivars in the in vitro generated plantlets (Loutfi, 1999). Such contradictory counts and inconsistent investigations are hampered by the absence of soft tissues in mitosis, especially from adult palm trees (Loutfi, 1966) and may also be attributed to the numerous and small-sized chromosomes.

Cytogenitical studies to firmly establish the chromosome number and karyotype in order to recognize chromosomal aberrations and to achieve early detection of gender in date palm seedlings resulting from seed germination, are highly desired. Expansion of the date palm Gene Bank and biodiversity management using molecular tools represent important goals. The application of in situ hybridization (ISH), fluorescent in situ hybridization (FISH), spectral karyotyping (SKY), and other techniques, to identify individual chromosomes and karyotype, and to detect chromosomal aberrations and clonal variations, should also not be ruled out. Although Sijak Yakovlov et al. (1996), based on chromomycin staining technique, have detected the presence of sex chromosomes carrying distinctive nucleolar heterochromatins that help detect the gender, nevertheless, rather simpler tools for early detection of gender in date palm are of great importance. In a preliminary study of Al-Ani and Abdullah (1987) on microsporogenesis in some male cultivars of date palm, namely, 'Green Ghannami' and 'Red Ghannami', 18 bivalents were observed at M1. However, in a few microsporocytes an early disjunction of one pair of the 18 bivalents was recognized before the rest of the complement at early anaphase of the first meiotic division (A1). Such observations have been interpreted by some cytologists as an evidence for the presence of sex chromosomes in date palm. However, this needs to be confirmed and supported by specific gene markers or application of the FISH staining technique to firmly distinguish the X chromosome from the Y chromosome.

Al Salih et al. (1987b) in one male cultivar ('Ghannami Akhdar') counted 9 bivalents in microsporocytes and 18 chromosomes in the root. The latter reference contradicts with all available references that deal with chromosome number in male date palm (Table 1).

Through personal communication, professor Alwan pointed out that he and his M.Sc. student at Basrah University in 1991 have counted 18 pairs of homologous chromosomes and have detected an X and Y sex chromosomes in some male date cultivars. Furthermore, they have encountered some chromosomal aberrations and polyploidy (pers. commun. with Prof. Dr. A.A. Alwan, 10 Feb. 2010).

Some careful cytogenetic work is needed to construct solid information on the normal date palm karyotype, in order to establish a basic reference for studying chromosomal aberrations, gene mutations and polyploidy in this crop. This will help the researcher detect the cytogenetic nature of various kinds of clonal variations and chimerism, especially in those that appear during various stages of micropropagation, and in induced mutations by chemicals or radiation. Hence, microsporogenesis and pollen formation; megasporogenesis and embryosac formation; and mitosis in meristematic cells at root apex should be the important target for karyotpe analysis.

In the present article we will consider the subject of karyotypic analysis and the criteria used for karyotyping, while the process of microsporogenesis and pollen formation will be treated in some detail in another article, with some emphasis being directed towards this phenomenon in the date palm.

KARYOTYPE AND KARYOTYPE ANALYSIS

The term karyotype is used to designate the number, size and shape of chromosomes of a somatic cell, of an individual belonging to eukaryotes, arranged in a standard manner.

A number of systems have been used to designate each homologue in the chromosome complement of any species, be it a plant, an animal or any other eukaryote. Some systems designate the chromosomes by Roman numbers, others use Arabic numerals, others use letters, and still others use both Arabic numerals and letters, to designate individual chromosomes, or groups of chromosomes which share certain characteristics, as in the human karyotype (Figs. 1, 2, 3 and 4).

Blakeslee (Avery et al., 1959) in *Datura stramonium* L., designated each one of the twelve pairs of chromosomes by two Arabic numerals. So each chromosome of the longest homologue is designated 1.2; the second 3.4; the third 5.6 and so on; while the shortest homologous chromosomes are designated 23.24 each (Figs. 2 and 3). In this system, the two Arabic numerals stand for the long and short arms of the chromosome respectively, while the full stop in between, represents the centromere that separates the two arms from one another. Blakeslee's system of designating chromosomes in *Datura* was aimed at recognizing translocations that occur between non-homologous chromosomes, as well as some other abnormalities that involve whole arms such as the formation of isochromosomes. However, this system did not gain wide usage by cytogeneticists, and some other systems have been applied in studies of the karyotype of most other species of eukaryotes.

The most commonly used system in karyotypic analysis is the one that designates each one of the two chromosomes in any homologue by one figure that is indicated by one Arabic numeral, as will be discussed below. Here, too, the two arms are, in turn, designated by specific letters that stand for long and short arms of the chromosome.

Criteria for Karyotype Analysis

The most important criteria used in cytogenetics to analyze the karyotype in somatic cells of any individual that belongs to eukaryotes, may be summarized in the following:

1. Chromosome Length. As a rule, the longest chromosome is given number 1, the second number 2, and so on. For example in onion, *Allium cepta*, where 20 chromosomes are present in somatic cells, the chromosomes are arranged in the karyotype in 10 pairs, or ten homologues. The longest pair is given #1, and the shortest homologue is designated as #10. In *Datura stramonium*, somatic cells contain normally 24 chromosomes, arranged in the ideogram of the karyotype from the largest to the smallest homologue (Figs. 2 and 3).

Similarly, a normal human somatic cell contains 46 chromosomes representing 23 pairs (or 23 homologues). Of these, 22 homologous chromosomes are called the autosomes and the remainder homologue is the sex chromosome. Both males and females of humans have 22 pairs of autosomes, but they differ in the sex chromosomes. While the female has two X chromosomes (XX), the male has one X and one Y (XY), in addition to the 22 pairs of autosomes in each.

Thus, the karyotype of human is represented by idiograms such as those indicated in Figure 4.

Here, in addition to the Arabic numerals designating each homologue, the autosomes are grouped into 7 groups designated by letters: A, B, C, D, E, F, G; while the sex chromosomes are placed at the end in a separate group. Both the Arabic numerals and the group-designating letter, in addition to some other abbreviations, are used when referring to any chromosomal aberration or syndrome. For example, the cytogenetic formula for Down's Syndrome (or Mongolism) which is characterized by having 3 copies of chromosome number 21, is expressed as: 47, XY Trisomy 21 G if the mongoloid person is a male and 47, XX Trisomy 21 G if the syndrome occurs in a female.

Regarding the karyotype of date palm, *Phoenix dactylifera* L., on the bases of

2n=2×=36; and 1n=1×=18; the largest homologue is given the Arabic numeral 1, and the shortest homologue is designated as chromosome number 18; while the remainder homologues lay in between, in a decreasing sequence of size, and increasing sequence of numbers (Fig. 1).

It has been consistently noted in may date cultivars that 4 or 5 pairs of homologous chromosomes are relatively larger than the remainder chromosomes in the complement, and the same size variation agrees with that observed in meiotic metaphase of the first division (M1) in the microsporocytes as observed in anthers of staminate flowers in male spadex.

Chromosome length is measured in units of micrometers (μm) and the measurements can be calibrated in the microscope by means of an ocular- and stage-micrometers. This helps the researcher determine the length of any chromosome at any stage and at any specific magnification.

As a rule, the chromosomes of plants are usually larger than those of animals; and the chromosomes of monocotyledons are generally larger than those of dicots. However, the chromosomes of *P. dactylifera* are among the exceptional examples of monocots having relatively small-sized chromosomes.

In date palm, the chromosome length variation lays between less than one micrometer to less than 3 micrometers. According to Al Salih and Al Rawi (1987) in their study on the karyotypes of two female cultivars of date palm, namely 'Ashkar' and 'Liwi', they have reported the following ranges of variation in chromosome lengths in the root tip of the studied cultivars as observed in seedings' root apices. In 'Ashkar', the longest chromosome (#1) was 2.389 micrometer and the shortest chromosome (#18) was 0.746 micrometer. In this cultivar, they counted 2n=36 chromosomes and have encountered the following chromosome number variations: 32, 34 and 36.

In the 'Liwi' cultivar, however, the longest chromosome (#1) was 2.552 and the shortest (#16) was 0.99 micrometer; and the variation in chromosome numbers in 'Liwi' was more pronounced, and was as follows: 28,32 34, 36 and 64.

In another study on roots from seedlings obtained from seeds belonging to another 2 female cultivars of date palm, namely, 'Sayer' (an early cultivar) and 'Khsab' (a late cultivar), Al Salih et al. (1987) counted 2n=32 chromosomes in 'Sayer', with variations in chromosome number range: 32, 34 36. In 'Khsab' however, they reported 2n=36 with a variation range in the number of chromosomes comparatively smaller, as they found 36 and 32 chromosomes in metaphase of cells of root apical meristem, with the majority of cells, revealing 36 chromosomes (Table 1 and Fig. 1).

2. Position of Centromere. The two exact copies of DNA that make up each chromosome are called chromatids. The two chromatids of each chromosome are attached to a point called the centromere, which is also called the primary constriction. Normally, each chromosome has one centromere (monocentric) and the position of centromere in each one of the two chromosomes of any homologue is identical. The centromere divides the chromosome into two arms; a short arm, called the p arm; and a long arm called the d arm. In human cytogenetics, the short and long arms are designated as p and q arms respectively.

The value obtained by dividing the long arm (p) by the short arm (d or q) is called an arm ratio. Depending on the value of arm ratio the chromosomes are described as shown in the following Table 2.

No matter what terminology is applied, the occurrence of pericentric translocation may result in the formation of an acentric chromosome which lacks the centromere; and a dicentric chromosome with two centromeres. Acentric chromosomes are usually lost soon after its formation, and the dicentric chromosome also causes chromosomal anomalies, and has specific configuration when observed in mitosis or meiosis, and is not perpetuated through later cell generations.

Prior to the 1960's, when Moorhead and Nowell described the Giemsa stain in their chromosomal preparations, conventional cytologic stains such as acetocarmine, aceto-orcein, gentian violet, hematoxylin and Feulgen stains have been used to stain

chromosomes. Such conventional techniques were used to uniformly stain chromosomes and leave the centromere constricted; hence, enabling the cytologist to measure chromosome length, position of centromere and to determine the arm ratio.

At present, more efficient staining techniques are used to highlight some structures in the chromosome such as the centromere, telomeres and nucleolar organizers.

3. Nucleolar Organizers or Secondary Constrictions. Each nucleus of the eukaryotes has one or more distinct structures where ribosomal RNA (rRNA) is synthesized. These structures are parts of the nucleus. Each nucleus contains at least one nucleolus which appears as a dense, distinctly stained area. The number of nucleoli in the nucleus vary from 1 to 10, and each nucleolus is determined by a specific region on specific chromosome(s) called the nucleolar organizer. The number of nucleoli in each nucleus is supposed to be the same as the number of nucleolar organizers characteristic of the individual.

Hence in gametic cells and other haploid cells, there must be at least one chromosome with a nucleolar organizer. In somatic diploid cells, there should be at least 2 nucleolar organizers (present on both chromosomes of a given homologue) or any other even number of nucleolar organizers (2, 4, 6, 8 or 10). However, usually one or few nucleoli may be seen per nucleus as a result of fusion of a number of nucleoli at the interphase, as they become associated together forming a bigger, densely stained mass or masses.

In human, nucleolar organizers are present in some homologous chromosome, and in *Datura*, *Allium*, *Zea maiz*, there are nucleolar organizers on specific homologous chromosomes (Figs. 2 and 3). In *Datura* nucleolar organizers or secondary constrictions are present in 7 homologous chromosomes.

Unfortunately, in the date palm, and to the knowledge of the authors of this article, no reference is available that indicates the presence of such structures, or the homologous chromosomes that carry nucleolar organizers.

4. Satellites. These are spherical or ovoid regions recognized in some homologues, and are located at the end of the chromosome, and separated from the rest of the chromosome arm by a tiny thread-like link of chromosomal DNA. These constricted regions of the chromosomes are secondary constrictions located in specific chromosomes near the terminal portion of the arm, thus, resulting in the formation of a satellite.

In *Datura* 6 homologous chromosomes do have satellites (Fig. 3) and in human 5 homologous chromosomes have satellites The latter are present in chromosomes #13, #14, #15 of the D group; and in chromosomes #21, #22 of the G group (Fig. 4). The chromosomes that carry satellites can easily be distinguished from the remainder homologues of the complement. The presence or absence of satellites helps the researcher recognize some specific chromosomes, and enabled cyto-geneticists to associate certain syndromes to specific chromosomes, and to help establish what is called the cytogenetic map.

5. Banding Pattern. The use of special staining techniques has revealed an intricate sets of bands (or transverse strips) on metaphase chromosomes of many different organisms. The position, size and number of bands are highly specific for each chromosome in the genome. Each chromosome can be accordingly, identified with ease. The unique pattern of banding characteristic of each chromosome has made it possible for human cytogeneticists to recognize chromosomal aberrations and to associate specific chromosomal abnormalities with specific diseases or syndromes (Lewis, 2003).

Examples of banding methods are:

- G-banding (or Giemsa banding).
- Q-banding (or Quinacrine mustard or Quinacrine dihydrochloride) which produces fluorescent bands.
- R-banding, or the Reverse Banding (Reverse of G- or Q-Banding).
- NOR Banding (Nucleolar Organizers Banding), and others.

AT-rich regions of DNA fluoresce brightly with Q-banding. G-banding is similar to Q-banding in that the AT-rich regions of the genome stain more intensively than those

of GC-rich regions. However, some other fluorescent stains that bind with GC-rich region have also been investigated. Such stains are best exemplified by chromomycine-A stain that highlights GC-rich regions of DNA, and thus, gives banding patterns just opposite (or reverse) to the pattern obtained by applying G-banding or Q-banding techniques. Such techniques that highlight GC-rich regions of DNA are called R-banding, as they give reverse banding patterns on chromosomes as compared to that obtained in G- or Q-banding techniques.

Chromomycine-A stain was found useful for detecting distinct nucleolar heterochromatins in male and female date palms, which will help the researcher distinguish the two sex chromosomes from each other and to determine gender, especially in young seedlings (Silijak Yakovlev, 1996).

Many other criteria and several techniques have been implemented in karyotyping such as autoradiography, in situ hybridization (ISH), fluorescent in situ hybridization (FISH), COD FISH, and spectral karyotyping (SKY). The SKY technique involves painting the two members of each homologous chromosomes with a distinctive color and thus, each chromosome in the genome could be identified, and many kinds of chromosomal aberrations can be recognized with certainty.

Unfortunately, progress in the field of cytogenetics techniques is rather limited in date palm research and some emphasis should be directed towards this issue.

CONCLUSIONS AND RECOMMENDATIONS

- The issue of chromosome number and karyotype of the date palm is rather heterogeneous and confusing.
- The diploid number of chromosomes reported ranged from 2n=26 to 2n=36, and in a single peculiar report on a male cultivar tree of date palm 2n=18 was reported.
- Table 1 summarizes some of the literature on the subject.
- All available studies on haploid (1n) were obtained from microsporocytes (PMCs) in anthers of flowers in male spadix.
- All references on haploid number reported 1n=18, as obtained in M1 of PMCs except one peculiar reference which reports 1n=9.
- While the diploid number revealed a wide range of variations, the haploid number showed a consistently identical number, i.e., 1n=18 with one negligible exception.
- The diploid chromosome number in most cultivars of date palm is 2n=36 chromosomes. Few cultivars, however, reveal other somatic diploid chromosome numbers. These include both plants raised from seed and those obtained from offshoots or through tissue culture.
- Variations in somatic chromosome numbers are not restricted to different cultivars of *Phoenix dactylifera* L., but may occur within the same cultivar and, in some instances, in somatic cells belonging to the same plant. The changes encountered, include aneuploidy (such as trisomy, monosomy and nullisomy); or euploidy (such as triploidy, 3n, tetraploidy, 4n, etc.). Nevertheless, such chromosomal variations in the date palm have neither been studied in detail nor have they been assigned to a specific chromosome(s).
- One of the problems associated with counting the diploid chromosome number has resulted from determining the chromosome number in mitotic metaphases of root tips obtained from germinating seeds and not from mitotic figures in root system of the mother plant. Therefore, these chromosomal counts and anomalies represent the status in the progeny, both in terms of chromosome numbers and genomic constitution, and do not strictly represent the female parent from which the seeds were obtained.
- Firm karyotypic analysis and precise chromosomal counts are urgently needed as a prerequisite to studies of genome project of date palm and for chromosome mapping, as well as determining the exact cytogenetic nature of clonal variations that may be encountered in micropropagation, or after exposure to chemicals or radiation.
- Implementation of some new cytological and molecular techniques such as the in situ hybridization (ISH), fluorescent in situ hybridization.

- (FISH), COD FISH, spectral karyotyping (SKY) and others, are highly requested in date palm to throw light on some cytogenetical problems, and to highlight the nature of chromosomal aberrations or genetic changes in somatoclonal variations that may occur in micropropagation techniques.
- Some of these studies may be included in the research programmes of staff members at UAEU, or as titles for projects to obtain an M.Sc. or Ph.D. as a partial requirement for offering higher degrees at UAEU.

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Tables

Table 1. Chromosome number and variations in some *Phoenix* species and cultivars.

Reference	Species or cultivar	Chromosome number 1n	2n	Variations and remarks
Nemec, B. (1910)	Non-specified cultivar (young embryo)		28	
Beal, J.M. (1937)	*Phoenix dactylifera*	-	36	No variations reported neither in haploid nor in diploid chromosome numbers.
	'Ammary'			
	'Barhee'	-	36	
	'Fard No. 4'	18	-	
	'Hallawi'	-	36	
	'Kristawi'	-	36	
	'Maktum'	18	36	
	P. canariensis	18	36	
	P. hanceana var. *Formusanum*	-	36	
	P. humilis	-	36	
	P. reclinata	-	36	
	P. syvestris	18	36	
Doulat, E. (1944) C.F. Darlington and Wylie (1955)	*P. dactylifera*	-	28	
Soliman and Al-Mayah (1973)	*P. dactylifera* (male cultivars)	18		
Al Salih and Al Rawi (1987)	'Lilwi'	-	32	28, 32, 34, 36 and 64
	'Ashgar'	-	36	34 and 36 Mitotic metaphase in roots of seedlings
Al Salih et al. (1987a)	'Sayer'	-	32	32,34,36 and 64
	'Khsab'	-	36	32 and 36 Mitotic metaphase in seedling root apex
Al Salih et al. (1987c)	'Ghannami Akhdar' (male cultivar)	9	18	No variations reported

Table 1. Continued.

Reference	Species or cultivar	Chromosome number		Variations and remarks
		1n	2n	
Selbi (1988)	*P. dactylifera* (male and female cultivars)	18	36	No variations reported
Al Ani and Abdulla (1987) P.C.	'Ghannami Red'	18	-	No variations reported in microsporo-cytes
	'Ghannami Green' (male cultivars)	18	-	
Loutfi et al. (1989)	*P. dactylifera* (female cultivar)	-	36	
Hussain et al. (1989)	'Barhi'	-	28	32, 34, 36, 42 and 64
	'Khadrawi'	-	28	28, 32, 36, 56 and 70
	'Khustawi'	-	36	26,28 (Root apex of seedlings)
Ibrahim et al. (1988)	*P. dactylifera* (female cultivar)	-	36	
Alwan (1991) (P.C) 2000	*P. dactylifera*	18	36	Chromosomal aberrations, aneuploidy and polyploidy detected
Loutfi (1999)	2 Moroccan cvs.	-	26	In the in vitro generated plantels

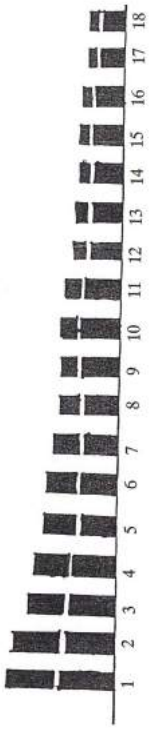
Table 2. Designation of chromosomes with respect to centromere position and arm ratio.

Arm ratio (long arm/short arm)	Chromosome designation
1 to <1.7	Metacentric
1.7 to <3	Submetacentric
3 to <7	Subacrocentric
7 to infinitive	Acrocentric
infinitive	Telocentric

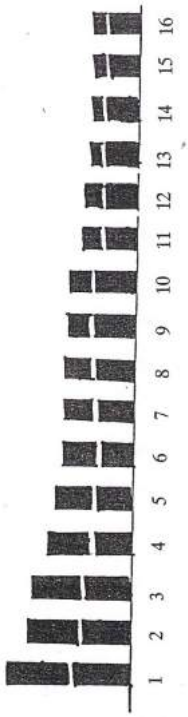
On the basis of mitotic metaphase counts obtained from root tips of seedlings raised from seed germination in two female date cultivars, Al Salih and Al Rawi (1987) reported the following results:

In 'Ashgar': 2n=38 (18 pairs)	In 'Lilwi': 2n=32 (16 pairs)
6 homologues were Metacentric	6 homologues were Metacentric
2 homologues were Submetacentric	2 homologues were Submetacentric
10 homologues were Subacrocentric	8 homologues were Subacrocentric

Figures



'Ashgar' with 18 pairs of chromosomes



'Lilwi' with 16 pairs of chromosomes

Fig. 1. Karyotype analysis of the date palm, *Phoenix dactylifera* L. (Al Salih and Al Rawi, 1987).

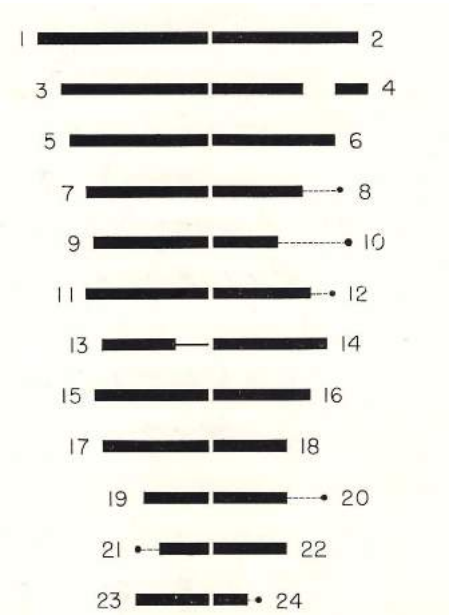


Fig. 2. Karyotype of *Datura Stramonium*, each chromosome is designated by 2 figures (see the text) (Avery et al., 1959).

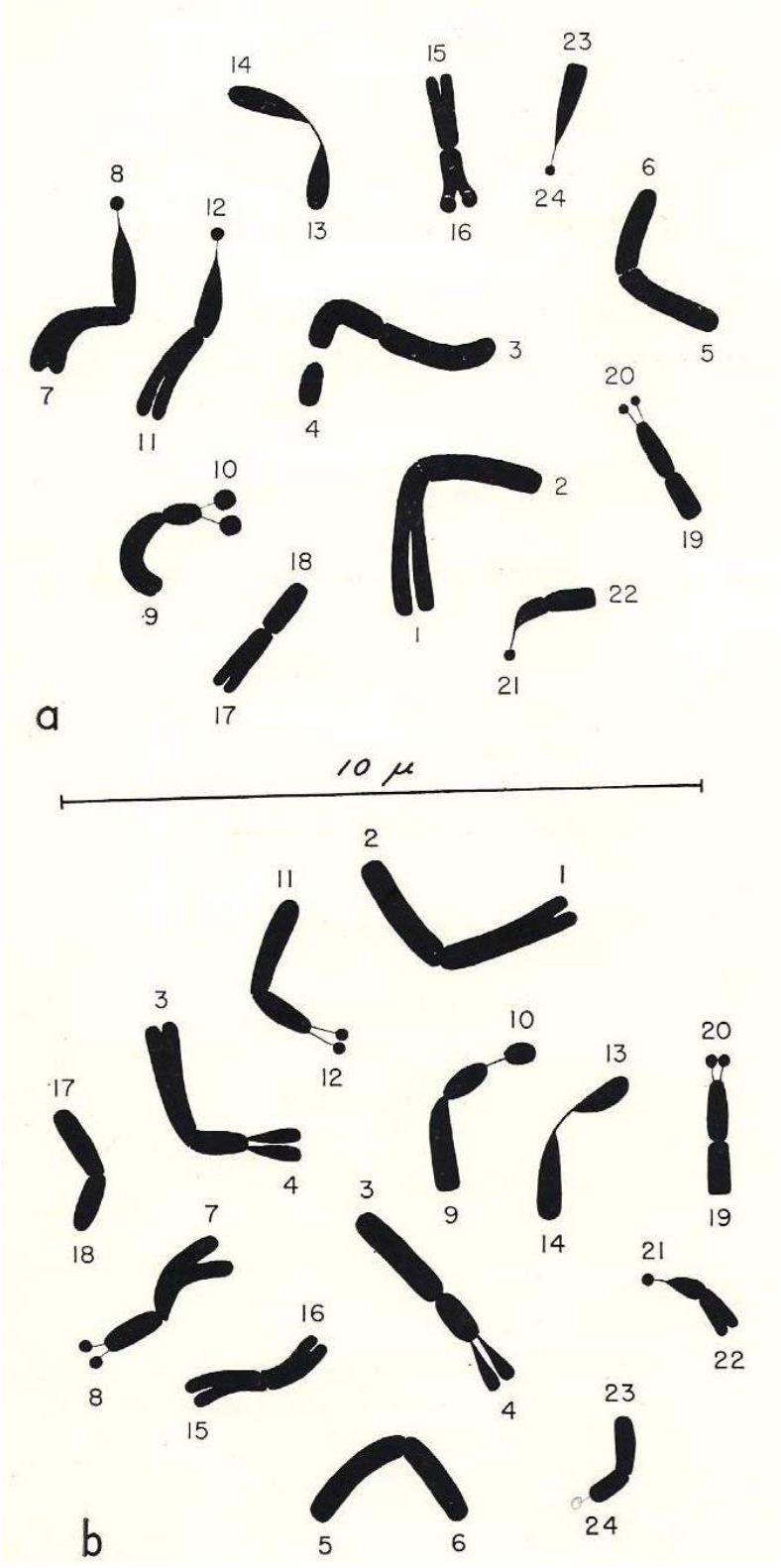
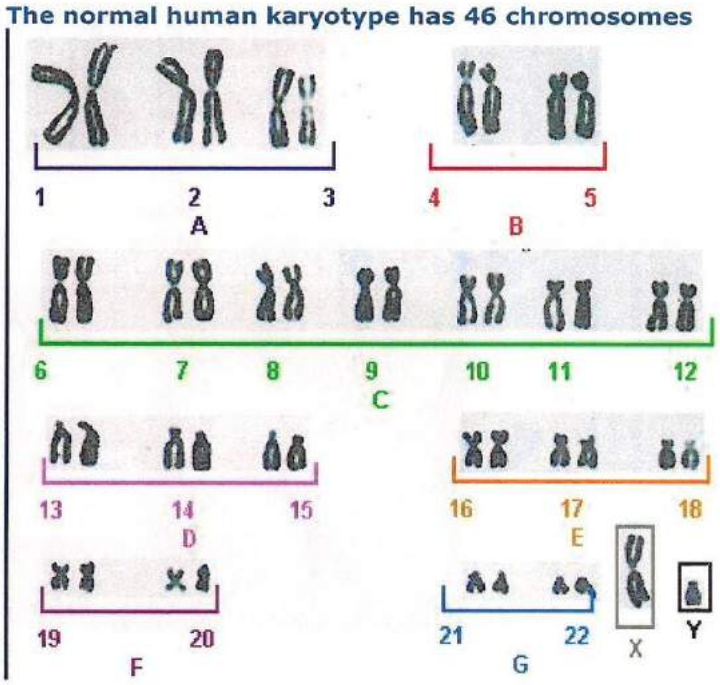


Fig. 3. Karyotype of *Datura Stramonium* 1n from a normal diployed plant; (b). 1n + 1 from a trisomic individual plant (Avery et al., 1959).

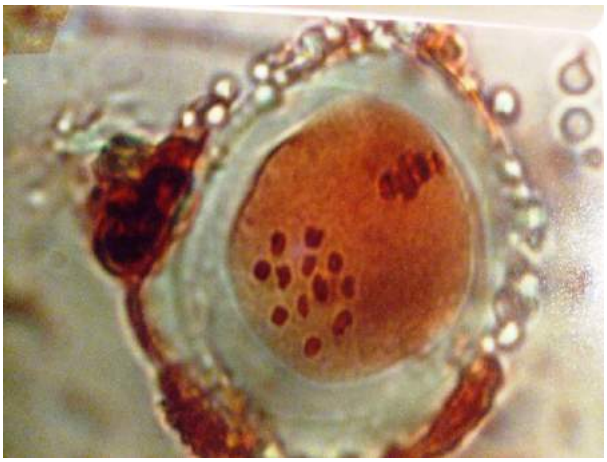


- 23 chromosomes derived from each parent
- 22 chromosomes are the autosomes
- 1 sex chromosome is derived from each parent
- Somatic cells have 46 chromosomes (22 pairs of autosomes and 1 pair of sex chromosome.
- XX is normal female; XY for normal male.

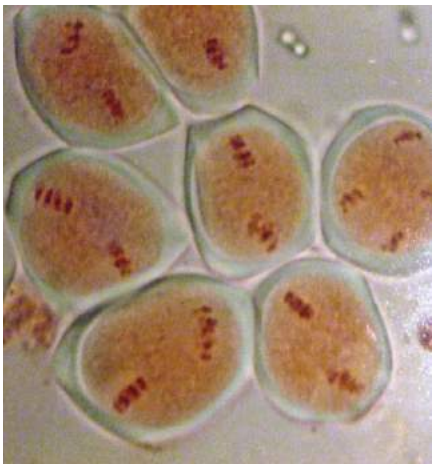
Fig. 4. Normal human diploid karyotype.

See high synchrony in PMCs at M1.

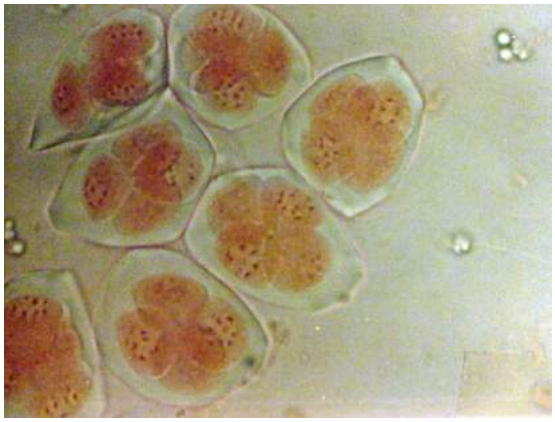
Fig. 5. Microsporocytes in *Trillium* showing five bivalents at metaphase 1 of the first meiosis (Erickson, University of Pennsylvania, 1960).



a. Metaphase 2 in *Datura* showing 12 chromosomes.



b. Metaphase 2 in *Datura* showing synchrony.



c. Microspore tetrads showing synchrony in *Datura innoxia*.

Fig. 6. Microsporogenesis in *Datura innoxia*, Mill. showing high synchrony (Al-Ani and Al-Okaily, 1990).

Development of 1000 Microsatellite Markers across the Date Palm (*Phoenix dactylifera* L.) Genome

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Keywords: *P. dactylifera*, microsatellite marker, simple sequence repeats, *adh* gene

Abstract

Date Palm is a major environmental and economic factor in arid climates in many countries around the world. Microsatellite markers have been proven to be very powerful in plant genome analysis because they are locus-specific, codominant, highly polymorphic and highly reproducible. In date palm only few microsatellite markers have been developed so far. Recently, the Cornell Medical College in Qatar issued a draft assembly of the date palm genome ('Khalas') generated by whole genome shotgun next generation DNA sequencing. In this paper, we analyzed the microsatellite motifs across the date palm genome. The results indicated that the most abundant type of microsatellite repeats are dinucleotide repeats (52442 motifs) followed by trinucleotide (28503 motifs) and pentanucleotide repeats (12873 motifs). The frequencies of tetra-nucleotide and hexa-nucleotide repeats were less across the genome (5555 and 5810 motifs, respectively). The most common type of dinucleotide repeat was GA (48.7%) followed by AT (37%). Out of 28645 trinucleotide repeats, TAA and GAA repeats were the most abundant repeats (28.1 and 27.1%) respectively. More than 1090 new microsatellite markers could be designed. The primary test for 50 primer pairs revealed that 28 (56%) were functional and 19 (38%) yielded polymorphic PCR products. We wish that the results of our study will be a starting point for researchers making use of the markers for genetic mapping and diversity analysis of date palm.

INTRODUCTION

Date palm (*Phoenix dactylifera* L, $2n=2x=36$), is a dioecious perennial monocotyledon fruit plant from the *Arecaceae* family. The predicted genome size was estimated to be approximately 250 Mbp (Barakat et al., 1999). The origin of this tree is Iraq, and recently, thousands of cultivars have been reported (Hanachi et al., 1998). Date palms have always been clonally propagated to ensure the identity and uniformity of the cultivars.

Discrimination among closely related cultivars by using morphological traits (including fruit morphology) are often unreliable and extremely difficult, especially because of the influence of environmental conditions (Elhoumaizi et al., 2002). Therefore, the need for using DNA marker technology for DNA fingerprinting has become increasingly important in recent years. Several marker systems have been used to study the genetic diversity of date palm, in brief, randomly amplified polymorphic DNA (RAPD) fingerprints have been used to identify date palm accessions in Algeria (Benkhalifa, 1999), in Morocco (Sedra et al., 1998), in Tunisia (Trifi et al., 2000), in Saudi Arabia (Al-Khalifah and Askari, 2003), and in Egypt (Soliman et al., 2003; Adawy

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et al., 2006). Amplified fragment length polymorphic (AFLP) markers have been applied to study the polymorphisms of date palm cultivars from Egypt and California (Cao and Chao, 2002; El-Assar et al., 2005; Adawy et al., 2006). Microsatellite or simple sequence repeat (SSR) markers have been used in plant diversity analysis because they are locus-specific, codominant, highly polymorphic and highly reproducible. Microsatellite markers have been developed and used to investigate genetic diversity in *P. dactylifera* (Billotte et al., 2004). They used $(GA)_n$ microsatellite-enriched library to develop 16 microsatellite markers. More recently, 17 microsatellite loci were developed by constructing two microsatellite enriched libraries of date palm by using $(GA)_n$ and $(GT)_n$ repeats (Akkak et al., 2009). These microsatellite markers have been used to assess the genetic diversity and relationships of date palm varieties in Tunisia (Zehdi et al., 2004), in Sudan (Elshibi and Korpelainen, 2007), in Oman (Al-Ruqaish et al., 2008), and in Qatar (Ahmed and Al-Qaradawi, 2009). However, for a wider use of microsatellite makers evaluating DNA polymorphisms in date palm tree, the development of hundreds of microsatellite markers would be necessary.

Unlocking the date palm occurred in April 2009, when researchers in the Weill Cornell Medical College in Qatar (WCMC-Q) used the variety named 'Khalas' (one of the most popular varieties of the fruit) to issue an assembly draft of the date palm genome generated by whole genome shotgun next generation DNA sequencing. The available sequence is a start point to apply advanced genomic technologies to a better understanding of date palm genome. The objective of this research was to study the frequency of microsatellite motifs across the date palm genome, and to develop new microsatellite markers.

MATERIAL AND METHODS

Sequence Analysis

The multi-fasta file of date palm sequence issued by WCMC-Q was downloaded from this web site address: http://qatar-weill.cornell.edu/research/datepalmGenome/download.html. The sequence file is named as (PdactyKAssembly1.0.fasta - 329328 KB) and contained 271804 fasta sequence clones.

Isolation of Microsatellites

The microsatellite motifs were classified as perfect, imperfect, compound perfect, or compound imperfect repeats according to the classification of Weber (1990), modified by Hüttel et al. (1999). A microsatellite is referred as 'simple', if a single type of repeat unit repeats several times (e.g., $(CA)_n$; etc.); a 'compound' microsatellite consists of stretches of more than one type of repeat unit (e.g., $(GA)_n{\cdot}(TA)_k$; $(GT)_k{\cdot}(TAA)_l{\cdot}(TA)_m$, etc.); a 'perfect' microsatellite does not contain mutations or interruptions (e.g., $(CA)_n$; $(TAA)_k$; $(CT)_m{\cdot}(GAA)_n$, etc.); an 'imperfect' microsatellite contains mutations or interruptions (e.g., $(CA)_nCC(CA)_m$; $(TA)_kAA(TA)_l{\cdot}(GA)_m$, etc.), subscripts k, l, n and m denote number of times the particular microsatellite motif repeats.

Short script was written by us in Perl software to collect the microsatellite motifs from the assembly draft of the date palm genome and categorize them as di-, tri-, tetra-, penta-, and hexa- nucleotide repeats. The percentage of accepted non-repeated nucleotides within the microsatellite motifs was fixed between 10-20% and called as error rate. For instance, in the following trinucleotide micosatellite $(TTA)_3$ –CAC – $(TTA)_3$ – GAC – $(TTA)_4$ – CCGG – $(TTA)_3$, consisted of 50 represent nucleotides, whereas ten (20%) nucleotides are not repeated sequence. However, only 10% of error rate was selected for further analysis in this study.

Plant Material

A total of 30 well-defined reference Iraqi date palm varieties were collected from two date palm stations belonging to the Ministry of Agriculture in Baghdad, Iraq. These female varieties are: 'Ashrasi', 'Barhi', 'Bream', 'Chipchab', 'Guntar', 'Helawi', 'Jamal

Al-Dean' and 'Khedrawi'. Total cellular DNA was extracted from young and healthy leaves as described by Rogers and Bendich (1985) with minor modifications. After purification, the obtained DNA was quantified and its integrity checked by using agarose gel electrophoresis (1%).

Primer Design and PCR Amplification of Microsatellites

Primer pairs were designed close to the microsatellite repeats in the flanking regions by using Primer3 v. 0.4.0 web base application available at this site address (http://frodo.wi.mit.edu/primer3/). The expected product size was limited to 200 bp, the length of the primers varied between 18 and 23 bases, the melting temperature (Tm) is fixed to be around 60°C, and all other parameters were kept as default values without change.

The PCR reactions were performed in a total reaction mixture of 20 µl containing: 50 ng of total cellular DNA (2 µl) as template, 1X PCR buffer (Roche, Manheim Germany), 0.2 mM of dNTP PCR mix (Roche), 0.5 U of *Taq* DNA polymerase (Roche) and 10 pmol of each primers (forward and reverse primers). Amplifications were performed in a Applied Biosystem Thermocycler (Applied Biosystem) with the following conditions: a denaturation step of 5 min at 95°C followed by 35 cycles of 15 s at 95°C, 15 s at 58°C and 30 s at 72°C, and a final extension step at 72°C for 5 min. Amplification products were separated on 8% polyacrylamide gels stained by ethidium bromide. The DNA banding patterns were visualized on an UV transilluminator and documented by using Gel Documentation System (Alpha Innotech).

RESULTS AND DISCUSSIONS

Microsatellite Motifs

The assembly draft of the date palm genome was analyzed and screened for microsatellites motifs using a script in Perl software (supplemented date). The results indicated that the draft sequence of date palm consisted of 321,278,327 bases including 94,386,304 adenine "A", 57,044,647 cytosine "C", 57,187,022 guanine "G", 94,100,785 thymine "T", and 18,559,569 unspecified nucleotides "N" (Table 1). Microsatellite motifs varied according to the three error rates (10, 15, 20%), less error rate showed less microsatellite motifs counted (Fig. 1). However, we used a 10% error rate in this study to increase the efficiency of microsatellite assessment.

Microsatellite motifs were frequently identified across the genome in about 105,183 microsatellite motifs (approximately one microsatellite per 3054.5 bases). Most of the repeats in date palm were of the simple/imperfect type (55,425 motifs) comparing to the simple/perfect (48,868 motifs). In contrast, many studies of plant species reported simple/perfect motifs to be the most abundant repeats in *Brassica napus* L. (Kresovich et al., 1995), in chickpea (*Cicer arietinum* L.) (Hüttel et al., 1999; Winter et al., 1999), and in lentil (Hamwieh et al., 2009). Among the microsatellite repeats, dinucleotide were the most abundant repeats across the date palm genome (52,442 motifs) followed by trinucleotide repeats (28,503 motifs) (Table 1). The most abundant repeat type in date palm genome was AG/TC (25,903 motifs) followed by AT/TA (20,160 motifs) then AC/TG (6,756 motifs). Billotte et al. (2004) developed 16 microsatellite primers from $(GA)_n$ microsatellite-enriched library by using GA. Later, Akkak et al. (2009) developed 17 microsatellite primers from two enriched libraries by using $(GA)_n$ and $(GT)_n$ repeats. Approximately 94.1% of their microsatellite motifs published were identified by the $(GA)_n$ library. This supports our finding that GA is the predominant repeat across date palm genome. However, the GT repeat is one of the most frequently occurring microsatellites in human and many mammals (Toth et al., 2000). This is also the case in some plant species, such as wheat (Varshney et al., 2000) and *Pinus radiata* (Smith and Devey, 1994), but this repeat (GT) is comparatively less frequent in other plants (Lagercrantz et al., 1993; Morgante and Olivieri, 1993). However, the dinucliotide repeats varied even within the genome itself between linkage groups or chromosomes. For

example, among the dinucleotide repeats across *Arabidopsis thaliana* genome, GT was most abundant in chromosome 1 followed by chromosome 4, 2 and 3 respectively, but this repeat was not found in chromosome 5 (Tamanna and Khan, 2005).

Trinucleotide repeat motifs have also been identified in plant genomes, the most frequently identified are $(TAA)_n$ and $(GAA)_n$ (Akkaya et al., 1992; Lagercrantz et al., 1993; Morgante and Olivieri, 1993; Winter et al., 1999). In the present study, trinucleotide repeats $(TAA)_n$ and $(GAA)_n$ were recovered in only 14,997 motifs of microsatellite representing 52% of the 28,645 trinucleotide motifs obtained across the date palm genomic sequence. Nonetheless, the isolation of trinucleotide repeats in date palm is expected to be important since the detection of polymorphisms involving trinucleotide microsatellite motifs may be easier compared with dinucleotide motifs, due to the presence of an extra base pair in the repeat unit (Hearne et al., 1992). Finally, less compound microsatellites were detected across the genome, included 702 dinucleotide motifs and 141 trinucleotide motifs.

Development of Microsatellite Markers

In total 1091 primer pairs could be designed in the flanking regions of simple/perfect microsatellite motifs. The expected sizes of these primers ranged between 113 and 345 bp with an average of 208 bp. Out of these primer combinations, 377 flanked dinucleotide, 352 primer pairs flanked trinucleotide, and 362 primer pairs flanked tetranucleotide repeats. Out of 33 published microsatellite primers only 22 (10 out of 16 microsatellite primers published by Billotte et al., 2004, and 12 out of 17 primers published by Akkak et al., 2009) could be detected in the sequence (Table 3).

To estimate the functional capacity of these primers, 50 primer pairs were tested with 8 Iraqi date palm varieties. The results revealed that 28 primers combinations were functional (56%) and 18 (36%) revealed polymorphic alleles (Table 4). If we extrapolate these results we would expect to obtain out of the 1091 primers at least 350 polymorphic microsatellite across the date palm genome. Certainly, these new co-dominant markers will be a starting point for researchers making use of the markers for genetic mapping and diversity analysis of date palm.

It is important to mention that clone number (>PdactyK1.0Scaffold_1817710_length_7070C) showed 91% similarity to the *Washingtonia robusta* alcohol dehydrogenase (*adh*) gene, (reference number on NCBI is: U65972.1). This microsatellite motif can be found under DPALM1091 (in the supplementary file). The *adh* gene was reported previously as the genetic basis for sex determination in date palm (Rajendran and Al-Mssallem, 2007). They yielded two clear bands of 800 bp and 1000 bp for the female genotypes and a single fragment of approximately 800 bp in male genotypes. In this study, we identified one microsatellite motif $(ATG)_2(AT)_3C(ATG)(AT)_3$ which is located 158 bases away from the *adh* gene. It needs to be tested if derived microsatellite markers could be used to screen for sex.

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Tables

Table 1. The number of the nucleotides and its frequency within the date palm genome.

Nucleotide	Count	Frequency
Adenine(A)	94,386,304	0.294
Cytosine(C)	57,044,647	0.178
Guanine(G)	57,187,022	0.178
Thymine(T)	94,100,785	0.293
Unspecific (N)	18,559,569	0.058
C+G	114,231,669	0.356
A+T	188,487,089	0.587

Table 2. Frequency of various types of microsatellite motifs observed across the date palm genome.

		Dinucleotide	Trinucleotide	Tetranucleotide	Pentanucleotide	Hexanucleotide
Simple	Perfect	24256	13182	3729	5449	2252
	Imperfect	27484	15180	1807	7409	3545
	Total	51740	28362	5536	12858	5797
Compound		702	141	19	15	13
Total		52442	28503	5555	12873	5810

Table 3. Date palm genomic clones used by Billotte et al. (2004) and Akkak et al. (2009) to develop microsatellite primers.

Reference	Primes	Clone number
Billotte et al. (2004)	mPdClR010	>PdactyK1.0Scaffold_271028_length_2103
	mPdClR025	>PdactyK1.0Scaffold_375496_length_3618
	mPdClR032	>PdactyK1.0Scaffold_952984_length_2335
	mPdClR050	>PdactyK1.0Scaffold_1723771_length_10937
	mPdClR057	>PdactyK1.0Scaffold_1698674_length_1883
	mPdClR070	>PdactyK1.0Scaffold_961639_length_1935
	mPdClR078	>PdactyK1.0Scaffold_36821_length_10487
	mPdClR085	>PdactyK1.0Scaffold_1084952_length_3573
	mPdClR090	>PdactyK1.0Scaffold_830856_length_5891
	mPdClR093	>PdactyK1.0Scaffold_1742274_length_2028
Akkak et al. (2009)	PDCAT10	>PdactyK1.0Scaffold_1871877_length_11760
	PDCAT11	>PdactyK1.0Scaffold_332319_length_10772
	PDCAT14	>PdactyK1.0Scaffold_1406391_length_898
	PDCAT15	>PdactyK1.0Scaffold_1377333_length_946
	PDCAT17	>PdactyK1.0Scaffold_1077978_length_376
	PDCAT18	>PdactyK1.0Scaffold_1165340_length_2957
	PDCAT1	>PdactyK1.0Scaffold_1498949_length_13938
	PDCAT20	>PdactyK1.0Scaffold_317589_length_3777
	PDCAT21	>PdactyK1.0Scaffold_836398_length_3971
	PDCAT3	>PdactyK1.0Scaffold_1661598_length_4767
	PDCAT6	>PdactyK1.0Scaffold_1657267_length_1165
	PDCAT8	>PdactyK1.0Scaffold_1541464_length_3258

Table 4. Forward and reverse primer sequences of the primers revealed polymorphic loci in mini core collection of Iraqi date palm varieties.

No	Primer Name	Forward Primer	Reverse Primer	Expected size	Tm°
1	DPALM100	GCCACTATCACCATTGCTGT	CAATGGAGGTCGTAGTGGTG	203	59
2	DPALM103	TTCCATCCCTGGAGAAAGG	AACCAAGACATCGTCCCAAG	200	60
3	DPALM104	GGAAAGTTTCGGAACATTTTGT	AACCCAACCTAAGCCCTACC	228	59
4	DPALM107	GGAAGGCGTCAAGGTATCTC	ACAACACGGGGAAAGAACAT	200	59
5	DPALM110	TGTCACATTATTTGAGCATAATCCA	ACCCTTTGTTGATGCACCTC	178	60
6	DPALM112	AGCAGGTTCATGGTTTGCTT	AGAACCAGGGAGGATGAGGT	200	60
7	DPALM113	GGTCCCGACGCCTATTTTAT	AGCAAAGTCCACCCCTTTTT	255	60
8	DPALM119	TGCGCTAAATAGTTCCCTTCA	CACATTCACAAGGCCTGCTA	208	60
9	DPALM120	TTCAATTCATCCCACTGCAA	CACCAACATGAGCAAATGGA	222	60
10	DPALM121	CATATGATTGTGATGGGGACA	CACCTCTCCGAGAAAACCAG	210	59
11	DPALM123	GGCAGGTGGATTGTTCTTGT	CAGGGGTATGGAGAGAGAGAGA	207	59
12	DPALM125	TTATGCTGAGGCCAGGTTTT	CATGCTGCAGAACCTGAAGA	191	60
13	DPALM132	TCAGCTCAAAGCACACAACA	CCGGAGATTTTGTTTCGATG	226	60
14	DPALM133	CAGATGGGATCGTTTTACCTG	CCGTATCGGGAGAGAGAGAG	197	59
15	DPALM139	TCTCGATCTCGACCTTGGTT	CGGATCCGGTTCTCTCATTA	202	60
16	DPALM141	CATTGCTCAGAAGCATCCAA	CTCTCCCTCCCTCTCGTTCT	212	60
17	DPALM142	CAATGGACCACCAAAATCAA	CTCTCCGAGAAAACCAGGTC	179	59
18	DPALM144	ACACACACACACACGCGAAT	CTTGCAGCCATTTAGGCAAC	187	61
19	DPALM146	ATGATTGAGAGGCAGGCAAA	GACAAGAGGGAAGGGGAGAG	198	60

Figures

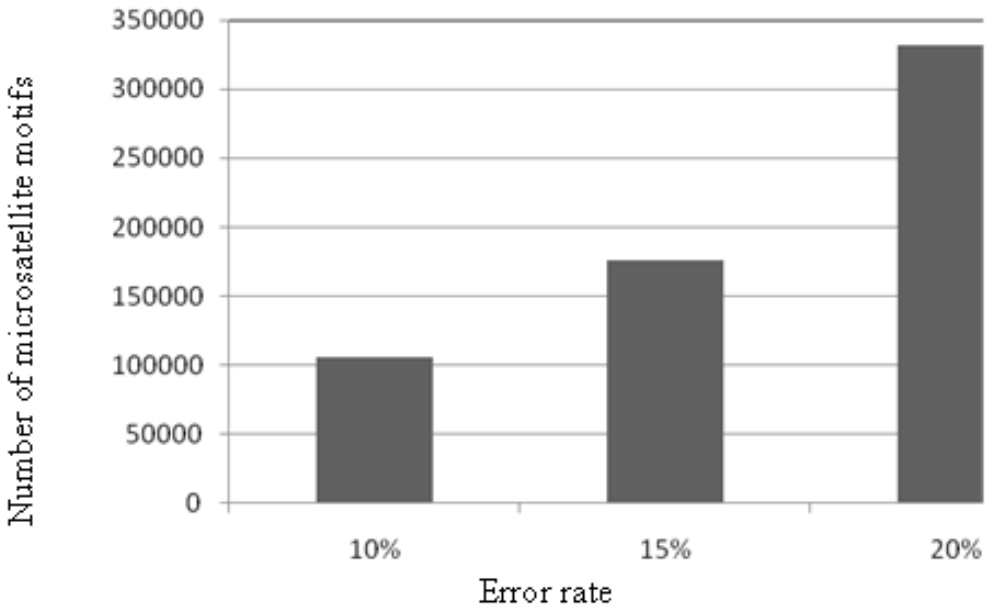

Fig. 1. Number of total microsatellite markers counted according to the different error rates (error rate: non-repeated nucleotides within the microsatellite motif). The figure shows that if more error in the microsatellite sequence is accepted then more microsatellite motifs could be counted.

Genetic Diversity of Date Palm Genotypes in Qatar as Determined by SSR and ISSR markers

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Keywords: date palm, SSR, ISSR markers, genetic diversity

Abstract

Date palm is the most important fruit tree in Qatar. Recently developed techniques, based on DNA markers, offer new tools for genetic analysis. The objectives of the present study are to analyze the genetic diversity among 15 different cultivars of date palm at the experimental farm of Qatar University using ISSR and SSR markers, and find out the genetic similarity and/or diversity among the well known date palm cultivars in the state of Qatar. DNAs were extracted from young fresh leaves. A total of 34 primers were tested for their ability to generate banding patterns in Qatari data-palm genotypes. However, 14 primers successfully produced clear bands in most of the studied genotypes. A total of 204 alleles were scored for all types of markers (SSR and ISSR). The similarity coefficient matrix was computed to cluster the data and to draw precise relationships among the fifteen studied Qatari date palm genotypes.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world. It is a dioecious, perennial, monocotyledon fruit tree that belongs to the family of *Arecaceae*. On a commercial scale, the Middle East and North Africa are the major date palm producing areas in the world. In Qatar, It is the most important fruit tree and is used as an ornamental or shade plant in parks, gardens and alongside roads as well. Date palm plantations represent 71% from the total area planted with fruit trees. The total area cultivated approximates 1366 ha (containing 335,765 trees bearing fruits and 146,955 non productive trees). Most cultivation is in the North and Middle area of the state where environmental conditions are favorable, soil has deep profile with low salinity compared with other parts of the country (Abufatih et al., 1999).

Date palms are generally propagated by separating the offshoots produced by individual trees. This method maintains the genetic integrity of date palm cultivars. Offshoots are produced in limited numbers during a date palm's life span (Zaid and de Wet, 2002). Seeds are breeding material with long backcrossing cycles. The first flowering of the trees takes place at the age of about 5-7 years (Baaziz, 2000; Zaid and de Wet, 2002). Therefore, the biological characteristics of date palm trees render it very difficult to compensate for the rapid decline of trees due to natural disasters. Extensive effort has been made to propagate date palms through tissue culture (Zaid and de Wet, 2002).

The characterization of date palm used to be done based mainly on fruit characteristics (e.g., shape, weight, color, aspect of skin, consistency and texture) and the morphology of leaves, spines. These characters are known to be strongly affected by environmental conditions and have limited discriminatory power. Accordingly, this has led to some cultivars with similar morphological characters being given the same varietal name.

Recently, developed techniques, based on DNA markers and polymerase chain reaction (PCR), offer new tools for genetic analysis and the construction of linkage maps. DNA markers are used to provide information on the relatedness of various clones or

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varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs.

To understand the genetic relationship among and within date palm varieties, RFLP, RAPD, SSR and AFLP markers have been used widely and efficiently to analyze the genetic diversity within and among date palm cultivars in many middle east countries such as Egypt (Soliman et al., 2003; Saker et al., 2006), Oman (Al-Ruqaish et al., 2008), Morocco (Sedra et al., 1998), Suadi Arabia (Al-Khalifah and Askari, 2003), Tunisia (Trifi et al., 2000; Zehdi et al., 2004a,b) and Sudan (Elshibli and Korpelainen, 2007).

Inter-simple sequence repeat (ISSR) is a PCR technique that uses repeat-anchored or non-anchored primers to amplify DNA sequences between two inverted SSR. ISSR markers are highly reproducible and provide highly polymorphic fingerprints (Bornet and Branchard, 2001). The random amplified polymorphic DNA (RAPD) technique as developed by Williams et al. (1990) is reliable, fast and easy for exploring genetic polymorphism within and among species. RAPD markers are based on the amplification of unknown DNA sequence using single, short and random oligonucleotide primers.

Microsatellites, or Simple Sequence Repeats (SSRs) are short stretches of repeated DNA, found in most genomes, which show exceptional variability in most species. This variability has made SSRs the genetic marker of choice due to their abundance, polymorphism and reliability compared to other types of DNA markers and for the vast majority of applications, including fingerprinting, analysis of genetic structure and for investigating evolutionary links between species and populations. Microsatellites regions are abundant and throughout the eukaryotic genome and highly polymorphic in length and are interspersed. However, it was only with the development of SSR markers for date palm (Billotte et al., 2004) that reliable, co-dominant and comparable molecular data on date palm populations could be generated.

The objective of this study is to use, ISSR and SSR markers to genotype the common date palm cultivars in Qatar and to assess the precise relationships between Qatari cultivars. This study will try to answer whether there is a genetic similarity or diversity among the well known Qatari date palm cultivars based on DNA markers.

MATERIALS AND METHODS

Plant Material

Fifteen Qatari Date palm cultivars derived from mature in vitro culture were used in this study (Table 1). These cultivars are now 10 years old and are grown in the Qatar University Experimental Farm at the North part of Doha. They are considered to be the most common date palm cultivars in Qatar. One tree representing each cultivar was chosen randomly and used for leaf sampling.

DNA Extraction

200-300 mg of fresh young leaf samples was grinded in liquid nitrogen with a cold mortar and pestle. DNeasy Plant Mini kit (Qiagen) was used to extract DNA from the Qatari date palm leaf samples according to the manual instructions of the kit (DNeasy Plant Handbook). Obtained DNAs were quantified and qualified by using agarose gel electrophoresis. Two µl of DNA from each sample were applied to 0.85% agarose gel and electrophoreses was done at 100 V for 30 min. The gels were stained in ethidium bromide and visualized under UV light.

ISSR Amplification

A total of 18 ISSR single primers were tested (Table 2). PCR reactions were performed in a total reaction mixture of 25 µl containing: 20-30 ng of total genomic DNA (1 µl) as template, buffer (GeneAmp, Applied Biosystems), 0.2 mM of dNTP PCR mix (GeneAmp, Applied Biosystems), 0.625 U of Taq DNA polymerase (AmppliTaq, Applied Biosystems) and 0.2 mM of primers. Amplifications were performed in a GeneAmp PCR System 9700 Thermocycler, with the following conditions: a denaturation

step of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 90 s at 44-60°C and 90 s at 72°C, and a final extension step at 72°C for 7 min. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed using the gel documentation system.

Microsatellites (SSR) Amplification

A set of 16 date-palm specific SSR primer pairs developed by Billote et al. (2004) (Table 3) was tested. PCR reactions were performed in a total reaction mixture of 25 µl containing: 20-30 ng of total genomic DNA (1 µl) as template, buffer (GeneAmp, Applied Biosystems), 0.2 mM of dNTP PCR mix (GeneAmp, Applied Biosystems), 0.625 U of Taq DNA polymerase (AmppliTaq, Applied Biosystems) and 0.2 mM of primers. Amplifications were performed in a GeneAmp PCR System 9700 Thermocycler, with the following conditions: a denaturation step of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 90 s at 52-60°C and 90 s at 72°C, and a final extension step at 72°C for 7 min. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed using the gel documentation system.

Data Analysis

Polymorphic bands were precisely measured by the gel documentation system software and scored for each genotype. Each reproducible polymorphic DNA band at particular position on the gel was treated as a separate character and scored as present (1) or absent (0) to generate a binary data matrix.

Data were then computed with the SPSS program to produce a genetic distance matrix which assesses the similarity between any two populations on the basis of the number of generated bands using Jaccard' s similarity coefficient (Jaccard, 1908). The matrix was then computed to draw phylogenetic diagrams.

RESULTS AND DISCUSSION

A total of eighteen ISSR were screened for their ability to generate consistently amplified band patterns and to access polymorphism in the studied varieties. However, only three ISSR primers revealed polymorphic and clear bands. The three ISSR primers generated two to eight polymorphic DNA bands with a range of 500 to 2500 bp. An example of the amplified products is shown in Figure 1. Among 16 SSR primer pairs tested for their ability to generate expected SSR banding patterns in Qatari data-palm genotypes, 10 primers successfully produced clear single bands in most of the studied genotypes. So far, six SSR primers did not amplify clear bands in our genetic materials even using different PCR conditions. The amplified SSR band sizes ranged from 100 to 300 bp. Figure 2 shows an example of an SSR band pattern that was obtained by primer 5.

A total of 204 alleles were scored for all types of markers (ISSR and SSR). Interestingly, 20 distinct unique SSR bands were obtained to represent 9 date palm cultivars. Four out of 7 different sizes bands obtained from primer 3 appeared in 'Hatamy', 'Barhee', 'Khadraway' and 'Thuri'. Each band represents one cultivar. On the other hand, some cultivars could be represented with single bands amplified with different primers. For example, 'Zahidi' was represented with single bands obtained from primer 4, 5 and 15 in 185, 302 and 155 bp, respectively. Six cultivars ('Hatamy', 'Helaly', 'Sheshy', 'Khadrawy' and 'Thuri') had two unique single bands from two different primers. However, one unique single bands were shown in 'Succary', 'Abu Main', 'Barhee', 'Naboot Saif' and 'Khanezy'.

Band pattern data were converted into binary data in an excel work sheet and were analyzed using the SPSS program to calculate similarity coefficient values according to Jaccard (1908). A similarity matrix between Qatari date palm cultivars showed an average similarity coefficient range from 0.000 to 0.750. The cultivars studied here were highly divergent at the DNA level. The highest similarity coefficient value was observed

between ‘Barhee’ and ‘Sultana’ (0.750) which seem to be the nearest two varieties and can be closely regrouped. The similarity coefficient value of 0.000 was obtained between ‘Abu Main’ and each of ‘Barhee’, ‘Sultana’, ‘Khush Zabad’, ‘Khanezy’ and ‘Thuri’, indicating how far the relationship is between ‘Abu Main’ and those cultivars. All the other cultivars displayed low levels of similarity but still were grouped with each other.

The Jaccard similarity coefficient matrix was computed to cluster the data and to draw the precise relationships among the 15 studied Qatari date palm genotypes. The dendrogram shown in Figure 4, illustrates the divergence between the studied Qatari date palm cultivars and suggests their tree branching.

‘Abu Main’ was in a separate far group compared to the rest of cultivars. ‘Barhee’ and ‘Sultana’ were very close to each other and could be considered one cultivar with a different name. The following cultivars: ‘Anbara- khadrawy’, ‘Khanezy-Thuri’, ‘Hatamy-Khalas’, ‘Zahidi-Helaly’ may constitute paired clusters (Fig. 3). Similar results were obtained previously by Ahmed and Al-Qaradawi (2009).

DNA markers are a powerful tool to provide information on the relatedness of various clones or varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. Simple Sequence Repeat DNA markers (SSR, or microsatellite markers) is considered the method of choice due to their abundance, polymorphism and reliability compared to other types of DNA markers. However, it was only with the development of SSR markers for date palm (Billotte et al., 2004) that reliable, co-dominant and comparable molecular data on date palm populations could be generated.

The highest levels of polymorphism for ISSR and SSR systems compared to other systems are also reported in previous studies (Belaj et al., 2003; Russel et al., 1997; Gomes et al., 1998; Maguire et al., 2002; Palombi and Damiano, 2002; Rajora and Rahman, 2003; Ferreria et al. 2004). This high level of polymorphism, associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage. Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion\deletion events, which generated the polymorphisms detected by RAPD analysis (Powell et al., 1996). The co-dominant nature of SSR markers also permits the detection of a high number of alleles per locus and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPD markers.

CONCLUSION

In this study, ISSR and SSR markers have been used to asses the molecular characterization and the phylogenic relationships of Qatari date palm cultivars. Our results provide evidence of a genetic diversity among the studied Qatari date cultivars and the ability of ISSR and SSR markers to detect the genetic diversity in date palm. We could conclude that all date-palm ecotypes are interrelated in spite of their agronomic divergence. Genetic similarities and dendrogram could re-group the Qatari date palm cultivars in a way that one cultivar (‘Abu Main’) was excluded from the group due to its dissimilarity with the other cultivars. Two cultivars (‘Barhee’ and ‘Sultana’) were much closer and could be considered as if they came from one origin. Some cultivars were grouped in different similar pairs. The polymorphic patterns obtained suggested that the ISSR and SSR procedures constitute alternative approaches that are suitable to examine the date palm’s genetic diversity at the DNA level.

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Tables

Table 1. Names of the studied fifteen Qatari date palm genotypes.

No.	Name
1	Zahidi
2	Hatamy
3	Helaly
4	Khalas
5	Succary
6	Anbara
7	Abu Main
8	Sheshy
9	Barhee
10	Sultana
11	Naboot Saif
12	Khadrawy
13	Khush Zabad
14	Khanezy
15	Thuri

Table 2. List of ISSR primers used in this study.

No.	Name*	Sequence	Ann. Temp
1	814	(CT)8TG	44°C
2	844A	(CT)8AC	44°C
3	17898A	(CA)6AC	44°C
4	17898B	(CA)6GT	44°C
5	17899A	(CA)6AG	44°C
6	17899B	(CA)6GG	44°C
7	HB 8	(GA)6GG	44°C
8	HB 9	(GT)6GG	44°C
9	HB 11	(GT)6CC	44°C
10	HB 12	(CAC)3GC	44°C
11	844B	(CT)8GC	44°C
12	HB 10	(GA)6CC	44°C
13	HB 13	(GAG)3GC	44°C
14	HB 14	(CTC)3GC	44°C
15	HB 15	(GTG)3GC	44°C
16	TA-1	(AG)10C	60°C
17	TA-2	(CT)10G	55°C
18	TA-3	(AGG)6	60°C

Table 3. List of SSR primers used in this study developed for date palm by Billotte et al. (2004).

Primer No.	Primer name	Motif repeat	Primer sequence (5'-3') F: Forward R: Reverse
Primer # 1	mPdCIR010	(GA)22	F: ACCCCGGACGTGAGGTG R: CGTCGATCTCCTCCTTTGTCTC
Primer # 2	mPdCIR015	(GA)15	F: AGCTGGCTCCTCCCTTCTTA R: GCTCGGTTGGACTTGTTCT
Primer # 3	mPdCIR016	(GA)14	F: AGCGGGAAATGAAAAGGTAT R: ATGAAAACGTGCCAAATGTC
Primer # 4	mPdCIR025	(GA)22	F: GCACGAGAAGGCTTATAGT R: CCCCTCATTAGGATTCTAC
Primer # 5	mPdCIR032	(GA)19	F: CAAATCTTTGCCGTGAG R: GGTGTGGAGTAATCATGTAGTAG
Primer # 6	mPdCIR035	(GA)15	F: ACAAACGGCGATGGGATTAC R: CCGCAGCTCACCTCTTCTAT
Primer # 7	mPdCIR044	(GA)19	F: ATGCGGACTACACTATTCTAC R: GGTGATTGACTTTCTTTGAG
Primer # 8	mPdCIR048	(GA)32	F: CGAGACCTACCTTCAACAAA R: CCACCAACCAAATCAAACAC
Primer # 9	mPdCIR050	(GA)21	F: CTGCCATTTCTTCTGAC R: CACCATGCACAAAAATG
Primer # 10	mPdCIR057	(GA)20	F: AAGCAGCAGCCCTTCCGTAG R: GTTCTCACTCGCCCAAAAATAC
Primer # 11	mPdCIR063	(GA)17	F: CTTTTATGTGGTCTGAGAGA R: TCTCTGATCTTGGGTTCTGT
Primer # 12	mPdCIR070	(GA)17	F: CAAGACCCAAGGCTAAC R: GGAGGTGGCTTTGTAGTAT
Primer # 13	mPdCIR078	(GA)13	F: TGGATTTCCATTGTGAG R: CCCGAAGAGACGCTATT
Primer # 14	mPdCIR085	(GA)29	F: GAGAGAGGGTGGTGTTATT R: TTCATCCAGAACCACAGTA
Primer # 15	mPdCIR090	(GA)26	F: GCAGTCAGTCCCTCATA R: TGCTTGTAGCCCTTCAG
Primer # 16	mPdCIR093	(GA)16	F: CCATTTATCATTCCCTCTCTTG R: CTTGGTAGCTGCGTTTCTTG

Figures

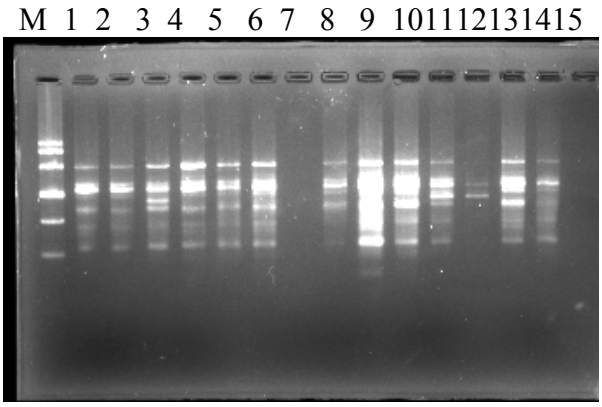

Fig. 1. An example of genotyping of 15 Qatari date palm cultivars using ISSR primer 1.

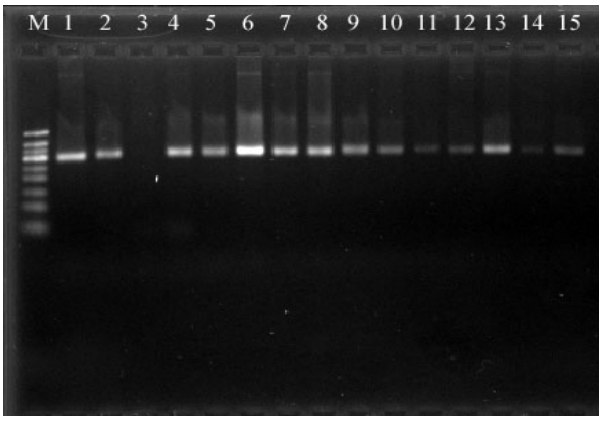

Fig. 2. Examples of SSR polymorphism banding patterns in a subset of 15 Qatari date palm genotypes using primers # 5. M: 50 bp. Standard ladder marker; Lanes (1-15): Qatari date palm genotypes described in Table 1.

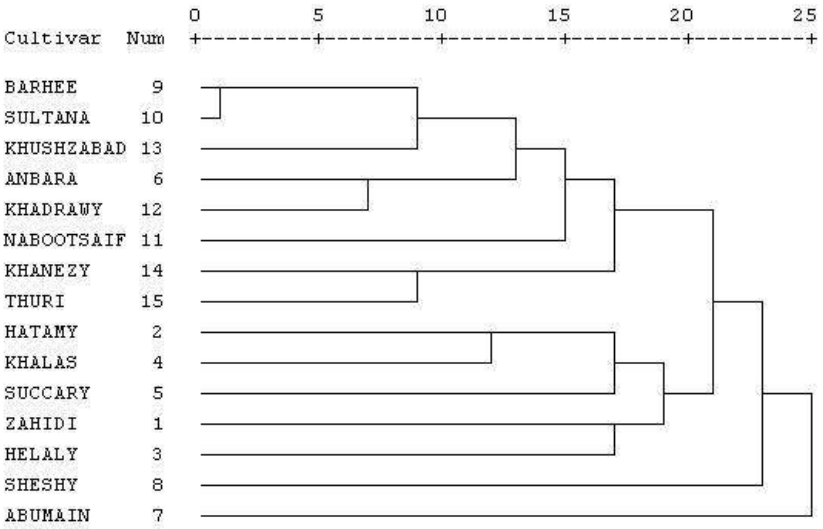

Fig. 3. Dendrogram of 15 Qatari date palm cultivars based on Jaccard genetic similarity coefficient using SSR data.

Date Palm Development Mission of Atul Ltd. in India

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Keywords: date palm, *Phoenix dactylifera*, tissue culture, cultivation, India, Atul

Abstract

Atul Ltd. has made long strides in translating its date palm dream - to transform India from the status of a major importer of dates to a major exporter of dates - through a mission mode project. It aims at the massive greening of the deserts of the Western border of India, using date palms, through systematic and scientific approaches, in a phased manner. A well knit strategy is adopted for the effective implementation of the project, on a participatory, public private partnership mode. There are five phases for the implementation of the project. The first part envisages the establishment of model demonstration plantations in key locations. A plantation consisting of seven superior varieties, has been established in an area of 100 hectares at Jaisalmer, Rajasthan. Farm development work on another 40 hectare site at Bikaner, Rajasthan is under progress. The second phase aims to mobilize quality planting materials of superior varieties, consisting of tissue culture plants, for the establishment of plantations. More than 47,000 primary hardened tissue culture date palm plants of four varieties have been imported from Arab nations and subjected to secondary hardening in the newly established nursery at Jodhpur, Rajasthan. The third phase is targeted at capacity building for the generation of tissue culture date palm plants in India, adopting the best protocol available. A joint venture company, Atul Rajasthan Date Palms Ltd. (ARDP), between Atul Ltd. and the Government of Rajasthan, has been established to set up a Tissue Culture Laboratory at Jodhpur. The objectives of the fourth phase include large scale scientific cultivation of date palms in the arid regions of Western India. The fifth phase envisages marketing of fruit and setting up processing industry.

INTRODUCTION

Atul Ltd., a member of the Lalbhai Group of companies, is one of the oldest business houses of India. Its registered office is in Ahmedabad, India and its corporate headquarters are located in Atul, Gujarat. It also has offices in the USA, the UK, Germany, China and Vietnam.

Date palm (*Phoenix dactylifera*) is believed to have been introduced to India by some Turkish sailors. Efforts to develop indigenous production of dates in India were initiated during the 1950s by the Indian Council of Agricultural Research. Some commercial varieties of date palm were introduced from the Middle East, Pakistan and the USA. The performance of some of these varieties was encouraging. Arid regions of north-west India, especially Kutch in Gujarat, Rajasthan and south-west Punjab have been identified as potential areas for date palm cultivation in India, besides selected regions in Tamil Nadu, in south India. Currently about 10,000 hectares of land is cultivated with date palm in India, more than 90% being in Gujarat. However, most of the plantations consist of just collection of cultivars/varieties raised from seeds or offshoots belonging to unknown varieties.

Considering the enormous potential of date palm in India, which is the leading importer of dates, Atul decided to support its scientific cultivation in the vast areas of arid lands merging with the Great Indian Desert, spanning over three major states viz., Rajasthan, Gujarat and Punjab. Within a short span of time, Atul has made long strides in

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translating its date palm dream through mission mode projects, transforming waste lands into greenery with a highly productive and widely acclaimed crop. It will have major impacts on the economic, social and cultural lives of the poor and marginal farmers of India.

MATERIALS AND METHODS

The date palm project of Atul in India is ambitious. It aims at the massive greening of the deserts of the Western border of India, using date palms, adopting systematic and scientific approaches, in a phased manner.

The striking feature of the project is its bearing on technology and scientific inputs. Date palm cultivation, largely, has not been scientific in majority of the areas where it is grown. Superior planting materials and scientific cultivation can boost up the productivity and quality of this crop. This fact is adopted as the base line in the projects of Atul. Benefits of the project will be alleviation of poverty and hunger, enhancement of rural employments, food security, women empowerment and eco-restoration.

A well knit strategy is adopted for the effective implementation of the project, designed on a participatory, public-private partnership mode. There will be five phases for the implementation of the project.

Phase I – Import of Secondary Hardened Plants and Setting Up Demonstration Farms

The first part envisages to establish model demonstration plantations in key locations to convince the farmers about the superior performance of date palms, propagated using tissue culture technique.

Phase II – Setting Up Nurseries, Importing Primary Hardened Plants and Hardening Them further, in India

The second phase aims to mobilize quality planting materials of superior varieties, consisting of tissue culture plants, for the immediate establishment of plantations. Since, date palm tissue culture plants are not available in India, Atul has adopted an 'all inclusive approach' with all the major tissue culture date palm suppliers in the Middle East and tied up with the Date Palm Research and Development Unit of the UAE University, Al-Ain, Al Rajhi Tissue Culture Laboratories, Saudi Arabia and UAE, Al-Wathba Marionnet LLC, Al-Ain and Green Coast Nurseries, Fujairah, for sourcing of planting material.

Phase III – Setting Up State-of-the-Art Tissue Culture Laboratory-cum-Production Unit with Overseas Technology

Phase three targets at capacity building for the generation of tissue culture date palm plants in India, adopting the best protocol available. Tissue culture propagation is an effective and efficient alternative for conventional vegetative propagation, to ensure rapid multiplication and establishment of true to type plants of choice varieties (Mohan Jain, 2007).

Phase IV – Acquisition of Land and Mass Propagation of Tissue Culture Date Palm Plants

Objectives of phase four include mass propagation and scientific cultivation of date palm in the arid regions of Western India.

Phase V – Marketing of Date Fruit Produce and Setting Up Processing Industry

This phase encompasses setting up co-operative societies which in turn will do buy back arrangements for purchase and marketing of date fruit with farmers.

RESULTS AND DISCUSSION

The arid regions (mainly in Rajasthan, Gujarat and Punjab) cover nearly 12% land

area of India. Of this, about 60% (13 million ha arable lands and 6 million ha wastelands) can be developed into clusters of date palms, with proper interventions and technical inputs.

Rajasthan, having the largest arid zone land, occupies almost 60% of the arid regions in India. This region has sandy soil of 8 to 10 pH, low rainfall and high temperature. This is further characterized with salinity, brackish underground water, strong wind erosion and low soil fertility which make the cultivation of crops difficult. However, date palm can withstand such extreme stress conditions. Rajasthan has the massive Indira Gandhi Canal Irrigation Programme which caters to more than two million ha. This makes Rajasthan and adjoining regions of Punjab and Gujarat ideal for cultivation of date palm in India.

Atul and the Government of Rajasthan have outlined a plan for tissue culture date palm cultivation in 2000 hectares in five years. Also, there is a long term plan of cultivating 1,00,000 ha in further 10 years. This is expected to generate a requirement of more than 15 million tissue culture date palm plants in India in the next 10-15 years.

More than 47,000 primary hardened tissue culture date palm plants of 'Barhee', 'Khalas', 'Khunezi' and 'Medjool' varieties (along with male plants) have been imported from Arab nations and subjected to secondary hardening in the newly established nursery at Jodhpur, Rajasthan. The nursery area is 10 ha and consists of more than 5000 m^2 of hardening facilities. The nursery is equipped with modern drip irrigation and fogging systems. The plants belonging to the varieties 'Barhee', 'Medjool', 'Khalas', 'Khunzi' and 'Madsari' male maintained for one year in the nursery recorded excellent growth (plant height: 54.5-75.0 cm; collar girth: 7.8-11.6 cm; number of leaves: 6.2-8.8; number of pinnate leaves: 1.3-2.6; length of the longest leaf: 33.1-49.3 cm; width of the largest leaf: 4.2-5.3 cm).

A plantation consisting of seven superior varieties, has been established in an area of 100 ha at Jaisalmer, Rajasthan, using tissue culture derived date palm plants of 'Barhee', 'Khadrawy', 'Khalas', 'Khunezi', 'Medjool', 'Saggai' and 'Zamli'. This includes the required number of male plants of 'Madsari' male and 'Ghannami' male also, for effecting pollination. Also, a specific demonstration farm has been established here. Modern techniques of farm development, irrigation and fertilization have been adopted. The irrigation is based on ground water source. Excellent growth was observed for the plants of the above varieties grown in the main field. Plants of the 'Barhee' variety recorded collar girth: 17.3 cm, number of leaves: 8.2, number of pinnae per leaf: 22.0 and length of the longest leaf: 63.8 cm, one year after field planting.

The importing of primary hardened plants and their further hardening before distribution to farmers, with Government subsidy will continue for further three or four years till phase three of this project is implemented to generate indigenously produced tissue culture planting material.

A joint venture company, Atul Rajasthan Date Palms Ltd (ARDP), between Atul Ltd. and the Government of Rajasthan, has been established to set up a state-of-the-art Tissue Culture Date Palm Laboratory at Jodhpur with overseas technology. This is a typical example of public-private partnership programme where the private sector brings in technology, co-operation and efficient management, while the Government supports the infrastructure and networking of farmers. Construction of the Tissue Culture Laboratory is in progress.

A Memorandum of Understanding was signed between the UAE University, Al Ain and Atul in presence of H.H. Sheikh Nahyan Mubarak Al Nahyan, Minister for Scientific Research and Higher Education, UAE to transfer technology of tissue culture of date palm to Atul.

Atul is planning to acquire vast areas of arid lands in Rajasthan and set up plantations of tissue culture date palm plants. Also, the Governments of the Western States of India have decided to expand the area under date palm cultivation. In this context, Atul has taken a major role to serve as a leader for date palm development in North West India. This will help create employment in rural arid regions, empower

women and reduce the impact of greenhouse gases. The joint efforts of Atul and the Government of Rajasthan shall help farmers to get date palm plants at a subsidized cost and encourage large scale cultivation of this crop. Atul shall provide training modules and other support services to the farmers for adopting the best scientific practices for date palm cultivation. Atul has developed a team of extension personnel, farm managers, scientists, academicians and marketing personnel for this purpose. Technical bulletins for promoting scientific date palm cultivation are being distributed to the farmers. Classes and field training are being organized at the demonstration farms..

Setting up of co-operative societies, which in turn will make buy back arrangements and marketing of date fruit, is aimed at the final phase. Infrastructure for collection of date fruit, grading, processing, packing, storage, logistics, branding, distribution and marketing would be established.

Atul is fully committed to make its dream of turning the dry deserts of India into high calorie green landscapes. It endeavors to involve all stakeholders in its journey to make this dream a reality.

CONCLUSIONS

India is the largest importer of date fruit. Atul has made substantial contributions to the date palm development programme in India. Atul is working in unison with all the key stakeholders to implement this unique project, in public-private partnership mode, wherein the strength of public sector and private sector are effectively converged for the project. The project is being implemented in five phases to have continuity and sustainability. It will help generate wealth in desert areas, empower rural population, particularly women and generate rural employment.

ACKNOWLEDGEMENTS

Thanks to the UAE University, Al-Ain and the Rajasthan Horticulture Development Society for supporting the date palm mission of Atul in India.

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Date Floating Ability: Adaptation to Regions with Floods

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Keywords: endocarp, gas trap, floating seed, flooding, floating dates, date palm fruit, cultivars, characteristics, varieties, disease resistance, Bayoud

Abstract

The geographic origin of date palms is unknown. Macrophotography and photomicroscopy were used to observe the structure and ability of the endocarp, whole fruit, or endocarp covered seed to float. We have found that the ripe fruit of dry fibrous low sugar varieties such as 'Degla Beida' can float on water. Soft high sugar or semi dry Bayoud-susceptible varieties, including the commercially important 'Deglet Noor' and 'Majhool', first sink but later are buoyed up by gases they produce before finally sinking again. Gas production by submerged fruit that would otherwise not float thus represents a characteristic varietal adaptation for seed dispersal suited to environments close to bodies of water or which are periodically flooded.

INTRODUCTION

Resistance to devastating diseases such as Bayoud, which wiped out 'Majhoul' dates from their country of origin, is considered to rely on finding resistant cultivars (Zaid, 2002; Mashaal and Obeidat, 2007). This entails knowing what cultivars exist and being able to distinguish between them (Mistiri et al., 1988; Benkhalifa, 1989; Al-Egaidi, 2010). Among the problems faced is having more than one name for the same cultivar, or having several cultivars with the same name (Benkhalifa, 1989). While biochemical and molecular biological methods are being tried; the traditional method relies on differences in morphological traits (Mostefai, 1994) including those of the fruit (Benkhalifa, 1989). It would be ideal to find easily distinguishable traits that correlate with the desired characteristic e.g., resistance to Bayoud.

The present work deals with distinguishing characters of morphological and ecophysiological significance, namely, the ability of the endocarp to trap air to float date seeds and the ability of soft high sugar cultivars to float upon submergence only after producing gas.

MATERIALS AND METHODS

An AT-1 Cannon camera with macro rings was used for macro photographs, and a Zeiss photo microscope with Nomarsky optics, was used for the micro photographs of the endocarp.

Dates of 'Degla Beida' and 'Deglet Noor' were bought in the Constantine and Algiers market. 'Majhoul', 'Deglet Noor' and 'Khodary' were obtained in Amman, Jordan.

Tap water in cups and bowls was used to observe floating of date fruit and seeds with attached endocarp. Evacuation was performed using a vacuum pump attached to a desiccator.

RESULTS AND DISCUSSION

We, along with Arab and other authors (Ali Ghalib, 1980; Ibrahim and Khleef, 1993; Zaid, 2002; Mashaal and Obeidat, 2007), did not have any trouble observing the membranous endocarp, "Qitmeer" in Arabic, which characterizes the fruit of the date palm. With our material we did not find that the endocarp "can be identified easily only in

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the early stages of fruit development" as reported by Fahn (1974).

Nomarsky optics surface photomicrograph (Fig. 1) of the endocarp layer of cells peeled from the fleshy mesocarp of unripe 'Deglet Noor' dates shows the longitudinal cells typical of analogous monocot leaf epidermis. As fruits ripen, the endocarp separates from the fleshy mesocarp as a membranous layer which usually remains attached to the stony seed. When the endocarp-covered seeds are placed in water they can float (Fig. 2).

The ripe soft high sugar or semi-dry fruit of Bayoud-susceptible varieties, including the commercially important 'Deglet Noor' and 'Majhool', unlike the dry varieties such as 'Degla Beida' do not float at first (Figs. 3 and 4). They sink, but beginning at one day after submergence individual fruit are buoyed up by gases they produce and float before finally sinking again (Al-Jamali, 1993). Fruit evacuated of gas sank but refloated again when enough gas was produced. Extra-dry 'Deglet Noor' and 'Degla Beida' fruit (not shown) floated immediately upon immersion in water.

Seeds of date palm do not float on their own. However, we found that the seeds can float, as mentioned in preliminary observations (Al-Jamali, 1998), in the presence of a membranous endocarp acting as a gas trap attached to them (Fig. 2). While floating, the date fruit or endocarp-covered seed can be carried with flood waters great distances from the mother date palm thus providing not only distinguishing varietal characteristics but a survival advantage for the species.

CONCLUSIONS

Date fruit and seed floating abilities vary with cultivars. These are characteristics that can be easily observed but are not sufficiently studied.

They may be added as potential indicators of other traits, such as, Bayoud-resistance, ability to survive without human intervention and as markers in the selection of future replacement cultivars of commercial importance.

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Figures

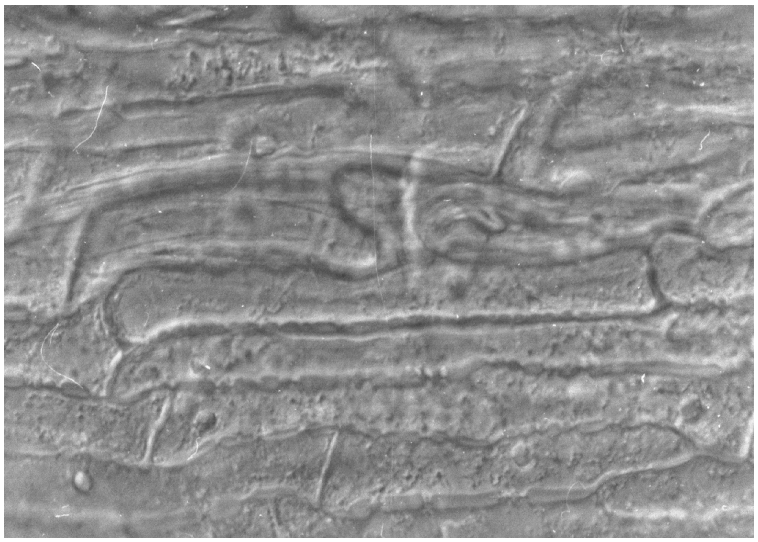

Fig. 1. Nomarsky optics photomicrograph of the endocarp layer of cells peeled from the fleshy mesocarp of unripe 'Deglet Noor' dates, showing the longitudinal cells typical of the analogous monocot leaf epidermis. As fruits ripen the endocarp separates from the fleshy mesocarp and remains with the seed (Fig. 2). With our material we do not find that the endocarp "can be identified easily only in the early stages of fruit development" as reported by Fahn (refer to text).

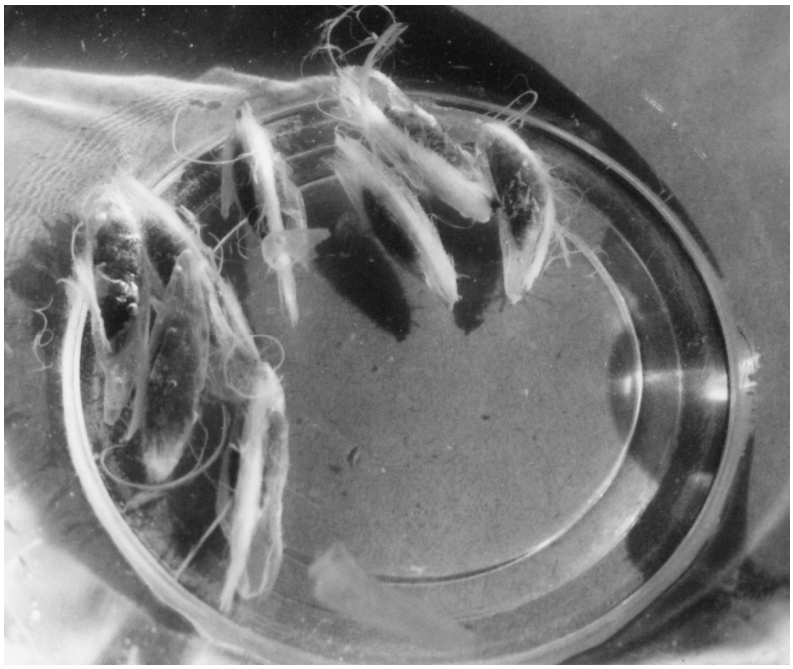

Fig. 2. The endocarp of fresh ripe 'Degla Beida' dates acts as a gas trap causing the seeds to float, thus permitting them to germinate some distance from the mother plant if there were water to carry them.

Fig. 3. Fruit of cultivars such as ‘Mejhool’ at left, ‘Khodari’ center and ‘Deglet Noor’ at right sink at first only to rise up when enough gases are trapped in their cavity to float them (e.g., ‘Majhoul’ at left). Some extra-dry individual fruits of ‘Deglet Noor’ (not shown) can float at first immersion.

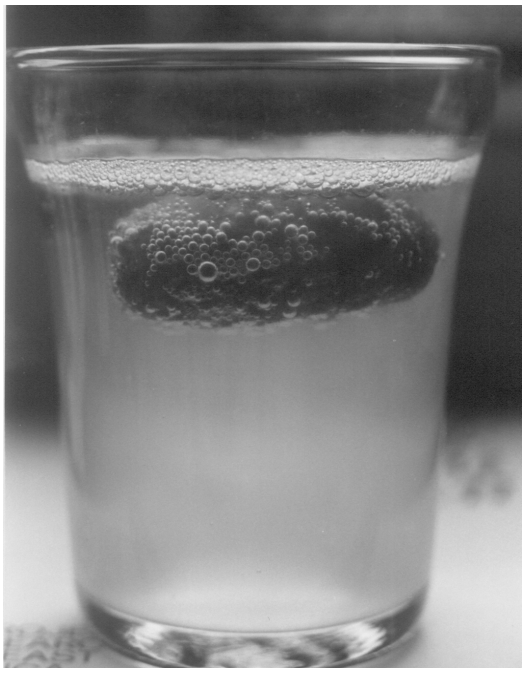

Fig. 4. ‘Deglet Noor’ here shown floating 3 days after first sinking. They will sink again if, either the gas in them is evacuated or in another week to 10 days time.

Xenic and Metaxenic Effect of *Phoenix pusilla* Pollen on Certain Date Palm Cultivars

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Keywords: *Phoenix pusilla*, date palm, pollination, metaxenia, xenia

Abstract

Pollinated female date palm flowers produce normal date fruits with a seed after fertilization. Un-pollinated flowers produce non-edible parthinocarpic fruits. Pollen from *Phoenix pusilla* which is closely related to the date palm fertilized the female date palm flowers of three date palm cultivars: 'Barhi', 'Majdool' and 'Sultana' which were experimented on. The female date palm trees were produced via tissue culture technology and maintained in the KISR date palm orchard, while plants of *P. pusilla* plants were raised from the seeds. The fruit characteristics of all the three cultivars pollinated by *P. pusilla* pollen were studied and compared with the fruits produced by the date palm pollen for three successive seasons from 2003-2005. The fruit characteristics such as fruit set, diameter, shape, color, maturity time, taste, flesh weight and yield per bunch were recorded. Seed morphology from the khalal, rutab and tamar stages of fruit maturation were recorded. The study showed that pollen of *P. pusilla* fertilizes the date palm flowers similar to the date palm pollen in all the three cultivars experimented. Fruit development was similar up to khalal stage in all the three cultivars studied. Significant variations in fruit and seed characteristics were noticed in the rutab and tamar stages. Fruit maturity was delayed when compared to the normal fruits and the fruits were seedless or with rudimentary seeds at maturity. Further studies of pollination with *P. pusilla* may lead to production of seedless dates and dwarf date palm hybrids.

INTRODUCTION

Date palm is a dioecious species, with staminate and pistillate flowers produced on separate trees. Female flowers are pollinated naturally by wind and effectively by hand pollination in commercial plantations. The female flower has three carpals, one of them develops into fruit after fertilization and the other two abort. The date fruit is technically referred to a drupe having fleshy mesocarp and hardy endocarp with seed. Normal date fruits are highly nutritious and edible. When the pollination fails, all the three carpels develop simultaneously into parthinocarpic fruits. These fruits are smaller than the normal fruits and non edible. They contain fibrous pulp with less sugar and rudimentary seedless endocarp.

Generally, pollens from male date palms were dusted on the female flowers for the normal fruit development in commercial plantations and home gardens. There are reports on direct pollen effect on date palm fruit physical and chemical characters (Swingle, 1928; Abdelal et al., 1983; Nixon, 1928, 1934). Pollens from other related species of date palm like *Phoenix reobelenii*, *P. reclinata*, *P. caneriensis* and *P. rupicola* were also successfully used to produce date fruits (Nixon, 1928, 1935). Recently, pollen of *Phoenix pusilla* (dwarf date palm) male was successfully used to pollinate female date palm cultivars (Sudhersan et al., 2009).

Fruit size and yield can be affected by various factors such as nutrition, climate, chemicals, bunch thinning and also by the genetic expression of the cultivars. In the case of inter-cultivar cross pollination, the genetic material derived from the pollen parent could have also an influence on fruit and seed characteristic features. This phenomenon is

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defined as xenia where the morphology of the seed is affected and metaxenia where the morphology of the whole fruit is affected (Sedgley and Griffin, 1989).

In date palm cultivation, pruning, pollination, fruit thinning, bunch removal and fruit picking are highly essential for good quality fruit production. The cost of date production increases when the tree grows longer due to high labour cost in many date producing countries. Mechanization is also expensive and unjustifiable in the case of small growers. Frequent climbing for fruit picking is highly dangerous in the case of taller old trees. The tree height is one of the major constraints in good quality date production. While attempting to develop dwarf date palm hybrids by crossing a dwarf species *Phoenix pusilla* with selected cultivars of female date palms at the Biotechnology Department of Kuwait Institute for Scientific Research (KISR), the authors have observed interesting pollen effect on date palm fruit characteristic features. The details of *Phoenix pusilla* pollen effect on three important date palm cultivars is presented herein.

MATERIAL AND METHODS

Plant Material

Phoenix pusilla called as dwarf date palm (Figs. 1 and 2) belonging to the same date palm family was introduced recently to Kuwait (Sudhersan, 2004) and three different female date palm cultivars propagated by tissue culture method growing inside the plant tissue culture orchard at the Kuwait Institute for Scientific Research (KISR) Shuwaikh campus were used as plant materials for the pollination experiments. The pollen from the male dwarf date palm was collected whenever flowering occurs in the dwarf date palm and stored at 1-5^{o}C under dry condition was used as pollen materials for pollinating the three selected female date palm cultivars. The fresh pollen mix collected from male date palm cultivars: 'Gannamy', 'Garvis', 'Diary' and unknown males was also used to compare the results with the pollen of the dwarf date palm.

Pollen Preparation

Mature male inflorescences were cut immediately after the breaking of the spathe and kept in paper bags and later transferred to a shady and moisture free area for drying. Spathe was removed carefully and bunches were spread over clean paper. Bunches were frequently changed from one paper to the other in order to avoid moisture logging. After 24h drying, the inflorescence strands were removed from the main rachis and again spread over clean paper for drying. All the male flowers were dried separately to avoid any pollen mixing. Dried pollens were collected in sterile dry containers which were sealed, labeled and stored at cool temperatures. A pollen mix was prepared by mixing the pollen of all the male date palms. Dried pollen mix and cotton balls were placed inside the sealed containers and shaken to infuse the balls with pollen during the experiments. These cotton balls containing pollen mix were used later to facilitate pollination.

Preparation of Female Date Palm Flowers

Female date palm cultivars 'Barhi', 'Madjhool' and 'Sultana' trees were identified inside the KISR Shuwaikh campus, Kuwait. The thorns around the female inflorescences of the selected cultivars were removed using a sharp pruning knife. After pruning the thorns around the selected unopened inflorescences, the spathe was removed using a sharp knife. From each of the 3 female date palm cultivars, 6 inflorescences were selected for the pollination experiment.

Pollination

The cotton balls infused with pollen from dwarf date palm and pollen mix from date palm cultivars: 'Gannamy', 'Garvis', 'Diary' and unknown male date palms were inserted inside the inflorescence individually without any pollen mixing between the dwarf date palm pollen and normal date palm pollen. Each inflorescence was opened manually for the pollination experiment. The pollen infused cotton balls were inserted

into the inflorescence and tied with a twine to form a cage around the cotton balls. After the insertion of the cotton ball with pollen, each individual inflorescence was completely covered with a paper cover to protect from contamination through the wind. The paper covers were removed after 15 days from the date of pollination.

Fruit Sample Collection and Observation

All the treatment and control bunches were carefully observed and the fruit characteristic features at different stages of fruit development: hababouk, kimri, khalal, rutab and tamar (Zaid and De Wet, 2002) were studied through the samples collected from the fruit bunches frequently till the fruit reaches maturity. The fruit weight, seed weight, seed size, fruit colour and number of days required for fruit ripening were observed and recorded. The readings were taken from 50 randomly selected fruits from bunches of each treatment and the mean value with standard deviation was calculated.

RESULTS

Metaxenia

The inter-specific pollination between date palms and dwarf date palm was successful and fruit set was noticed similar to the normal date palm pollination by known or unknown male date palm cultivars. *Phoenix pusilla* pollen fertilized the flowers of all the three cultivars experimented. The fruits from all the inflorescences pollinated by date palm pollen and dwarf date palm pollen grew, elongated and matured (Figs. 3-6). At the mature stage, the fruits developed through dwarf date palm pollen were different from the normal fruits in shape. The pollen from the dwarf date palm affected the fruit development and morphology at the stages of khalal and tamar. The size of fruit in 'Barhi' was a little smaller than the fruit size attained by normal date palm pollen, while in the other two cultivars, 'Madjhool' and 'Sultana', fruits were almost equal to the size of the normal fruit. The normal fruits produced by date palm pollen were oval in shape and the fruit produced by dwarf date palm pollen were dumbbell in shape. The mature fruits of the cross pollinated 'Sultana' and 'Madjhool' fruits were dumbbell in shape. The dumbbell shape was due to the aborted seed leaving a long seed cavity inside the fruit. The fruit characteristic features observed were tabulated (Table 1). In all the three cultivars studied, the pollen of *P. pusilla* delayed fruit ripening. About 15-20 days delay in fruit ripening was observed. There was no fruit colour change observed between the control and the treatment.

Xenia

Seed development was noticed in both treatment and the control at the initial stages. Later on the arrest of seed growth and development was noticed in the fruits developed by the dwarf date palm pollen. When the fruit developed by the dwarf pollen was cut into two halves, we noticed a seed cavity inside and a small rudimentary seed at the anterior end (Fig. 6). The normal seeds developed in date palm cultivar 'Majdool' were peculiar in shape having two wings (Fig. 7). The rudimentary seeds were small and similar to a grape seed in shape and size (Fig. 7). In the early stages, seeds showed embryo development, but the embryos were aborted in the ripened fruits due to the total arrest in endosperm development. A total of 50 fruits were cut in to two halves to study the endocarp and seed. In all the 50 fruits, we found rudimentary seeds at the anterior end of the endocarp. The rudimentary seed of 'Madjhool', 'Sultana' and 'Barhi', was 0.4, 0.3 and 0.3 cm respectively in length and 0.3, 0.2 and0.2 cm respectively in width. The seed weighed about 0.03 g in 'Madjhool', 0.2 g in 'Sultana' and 0.2 g in 'Barhi' (Table 1). The normal seed of date palm cultivar 'Madjhool' was 2.5 cm in length, 1.2 cm in width and 1.2 g in weight. The seeds collected at the khalal stage when cultured under in vitro conditions germinated and produced plantlets while the seeds collected from the ripened fruits failed to germinate due to the abortion of embryos at the fruit ripening stage.

DISCUSSION

Dwarf date palm pollen showed interesting variations on the fruit and seed morphological characters of the three cultivars: 'Barhi', 'Madjhool' and 'Sultana'. The seed size at the fruit maturity was 30 times reduced from the normal date seed produced by the normal date pollen.

In this trial, crossing was carried out between two species (interspecific) and both species were found to be compatible with each other and fertilization occurred in all the three species studied. However, seed development at the khalal stage was totally arrested due to endosperm formation.

Previous reports on such interspecific crosses revealed that pollen from *Phoenix reclinata*, *P. caneriencis*, *P. robelenis* and *P. rupicola* crossed with date palm for fruit quality improvement failed to produce better quality fruit, while the cross between the date palm and *P. sylvestris* produced slightly larger fruits than the normal (Nixon, 1935). In this present study using *P. pusilla* pollen fruits were without seed and were larger in size in the case of 'Madjhool' and 'Sultana', and of the same size in 'Barhi'.

In many inter-specific crosses, fertilization and early embryo development occurs but some irregular events subsequently take place, mainly the failure of the endosperm to develop properly resulting in embryo abortion and seed collapse (Ragavan, 1977). Similar results were obtained in this breeding trial with dwarf date palm. Seed development had begun at the kimri stage, however at the ripened stage only rudimentary seeds were observed at the upper end of the fruit.

Boyes and Thompson (1937) found shrivelled small seeds with floury endosperm in inter-specific crosses that failed to germinate. They attributed the difference in seed development to the chromosome imbalance in the endosperm. Brink and Cooper (1947) suggested that endosperm breakdown was the main reason for the failure in inter-specific and intra-specific crosses in plants. The concept of endosperm imbalance was proposed by Johnson et al. (1980) to explain endosperm development in inter-specific and intra-specific crosses and it was due to post fertilization incompatibility. Similar post fertilization incompatibility was observed in our inter-specific crossing in date palms.

Fruit development without fertilization is termed as parthinocarpy. Parthinocarpic fruits were reported in several fruit crops such as citrus, grapes, peaches, cherries, bananas and pineapple. Parthinocarpy occurs normally without pollination or stimulation by the pollen or induced by chemicals. Here in our study, the fruits are not true parthinocarpic but developed after fertilization. Initially the fruit developed with seed after fertilization by the dwarf date palm pollen and later on the seed development was arrested during the fruit growth due to the failure in endosperm growth. Finally, at fruit maturity a small rudimentary aborted seed was noticed at the anterior end of the endocarp.

Seed formation in an inter-specific cross is influenced by the mutual ratio of the chromosome number within the embryo, endosperm, and the tissue of the ovary surrounding it. This mutual ratio is disturbed in crosses between two different species. As a consequence of the altered mutual ratios between the chromosome numbers of embryo, endosperm, and ovary, morphological and physiological changes in the seed formation can occurr (Kuckuck et al., 1991). The current study supports the above approach. Although seed development was noticed initially, the breakdown of endosperm development was noticed latter on. Due to the development of disorders in the endosperm development, the embryo growth and development also ceased. Embryo culture techniques in vitro were carried out in our laboratory in order to rescue the hybrid embryos and get hybrid date palms (Sudhersan et al., 2009).

Seedless fruits have advantages over seeded fruits through longer shelf life and greater consumer appeal. Date fruit consumers have a liking for seedless dates because date seeds are hard and unpalatable. The naturally developed parthinocarpic date fruits are non edible and, the chemically induced ones are highly expensive and environmentally unsafe. However, the seedless date fruits developed through pollination by dwarf date palm were edible and economically feasible. The present finding could pave the way to produce edible seedless date fruits commercially. Perhaps this is the first report on the

seedless date fruit production using dwarf date pollen.

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Tables

Table 1. Xenic and metaxenic effect of *Phoenix pusilla* pollen on date palm cultivars.

Cultivar and fruit type	Fruit			Seed		
	length (cm)	width (cm)	weight (g)	length (cm)	width (cm)	weight (g)
			Madjhool			
Control	4.8 ± 0.3	3.1 ± 0.7	28.2 ±1.0	2.5 ± 0.4	1.2 ± 0.7	1.4 ± 0.4
Treatment	4.8 ± 0.7	3.0 ± 0.1	27.1 ±0.3	0.4 ± 0	0.3 ± 0.1	0.04 ± 0
			Sultana			
Control	4.6 ± 0.1	3.2 ± 0.4	29.7 ±0.7	2.7 ± 0.7	0.8 ± 0.1	1.3 ± 0.7
Treatment	4.7 ± 0.1	3.0 ± 0.3	30.2 ±0.1	0.3 ± 0	0.2 ± 0	0.02 ± 0
			Bahri			
Control	3.7 ± 0.3	3.8 ± 0.7	23.9 ±0.9	2.0 ± 0.8	0.7 ± 0.3	1.0 ± 0.7
Treatment	3.2 ± 0.2	3.3 ± 0.1	21.6 ±0.4	0.3 ± 0	0.2 ± 0	0.02 ± 0

± Standard Deviation; Control-Date palm Pollen Mix; Treatment-*Phoenix pusilla* pollen.

Figures

Fig. 1. Male *Phoenix pusilla*.

Fig. 2. Female *P. pusilla*.

Fig. 3. Date palm 'Sultana' fruit by *P. pusilla* pollen.

Fig. 4. Date palm 'Madjhool' fruit by *P. pusilla* pollen.

Fig. 5. Date palm 'Barhi' fruit by *P. pusilla* pollen.

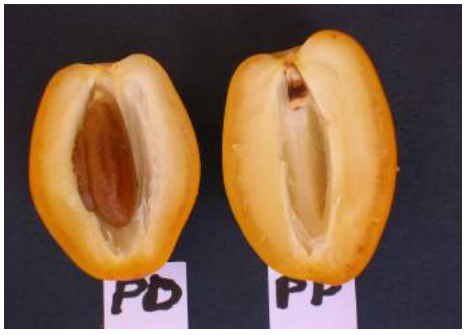

Fig. 6. Date palm 'Madjhool' normal fruit and fruit by *P. pusilla* pollen.

Fig. 7. 'Madjhool' seeds by normal and *P. pusilla* pollen.

The Effect of Arbuscular Mycorrhize (AM) Fungi on the Establishment of Date Palm (*Phoenix dactylifera* L.) under Saline Conditions in the UAE

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Keywords: Arabian Peninsula, ICBA, AM fungi, mycorrhizae, inoculum and salinity

Abstract

The date palm (*Phoenix dactylifera* L.) has great socio-economic importance in the UAE and the Arabian Peninsula. Research at the International Centre for Biosaline Agriculture (ICBA) has shown that date palms are obligatory dependent on arbuscular mycorrhiza (AM) fungi for growth under saline field conditions. One-year-old in vitro seedlings of the date palm variety 'Khenizi' were inoculated with commercial AM inoculum, BioMyc^{Vital} in a pot trial conducted at the ICBA plastic house. Plants were irrigated with fresh water as control and 5 and 15 dS m^{-1} salinity levels. Two fertilizer levels, full fertilizer level (15, 30 and 5 g/month/plant of NPK (20:20:20), compost mix and Osmocote (12:13:13+TE), respectively) and low fertilizer level which was 1/3 of the full fertilizer level were used as fertility treatments. The results showed significant differences among treatment means. However, no interaction among treatment factors was found. The mycorrhizal inoculum stimulated growth of date palm under all salinity conditions. Within 6 months, plant height and trunk diameter of plants inoculated with BioMyc^{Vital} was increased by 60.7 and 28.8% respectively, with fresh water whereas, 45.0 and 51.8% respectively with high salinity water (15 dS m^{-1}) irrigation compared to the non-inoculated plants. Interestingly, best growth was obtained at low fertilizer level. Inoculation coupled with low fertilizer level increased the plant height by 20.4% and trunk diameter by 18.4% over the full fertilizer level. The experimental data showed that date palms should be associated with AM fungi at nursery stage using the inoculants. Plants can then better withstand salinity stress and they are available for field transplanting in comparatively shorter periods saving up to one year in the nursery. The results also showed that date palms will grow better under natural conditions when effectively associated with AM fungi. Also, less chemical fertilizer and inputs are required to grow date palms when they are effectively mycorrhized. The implications of mycorrhizal inoculation with commercial inoculum for date palm production in UAE and in the Arabian Peninsula are discussed.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is widely grown in the Arabian Peninsula, North Africa, Middle East, America and Asia (Chao and Krueger, 2007). The area under date palm cultivation has continuously increased in the Arabian Peninsula during the recent decades and in UAE, for example, the number of date palm trees has reached to almost 41 million (MEW, 2005). World date production has reached to about 6.8 million metric tons of fruit (FAO, 2007). Date palm has great socio-economic importance in the Arabian Peninsula including the UAE due to its use for fruit production, ornamental, gardening and landscape purposes. Date palms are often grown under saline conditions. Salinity is a major concern for irrigated agriculture particularly in arid and semi-arid regions of the world including the Arabian Peninsula where survival and plant growth is limited only to salt tolerant species. Salinity has a major influence on plant growth and survival (Staples and Toenniessen, 1984). By inhibiting root growth, salt stress decreases the availability

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and uptake of water and nutrients and plant growth decreases (Delane et al., 1982).

Since harsh climatic conditions and soil and water salinity are major constraints for date palm production in the UAE, mycorrhizal symbioses could enhance their survival and growth. Arbuscular mycorrhizal (AM) fungi are the most ancient mycorrhizal type found in a wide range of environments. They occur under almost all natural communities and form symbiosis with over 80% of vascular plants (Smith and Read, 1997). The symbiosis confers numerous benefits to host plants including improved plant growth and mineral nutrition, tolerance to diseases and stresses such as drought, temperature fluctuation, metal toxicity and salinity (Al-Karaki, 2000; Al-Karaki et al., 2004; Borowicz, 2001). Mycorrhizal plants have more nutrient and water uptake from soil in comparison with non-mycorrhizal plants. The benefits of inoculating a wide array of agronomic plant species with AM fungi have been documented in numerous studies including date palms (Al-Whaibi and Khalil, 1994; Bouhired et al., 1992; Jaiti et al., 2007; Al-Karaki, 2000; Al-Karaki et al., 2004). Mycorrhizae and date palm symbiosis was reported in the Crescent desert of Iraq (Khudairi, 1969) and in the oasis of Qassim region of Saudi Arabia (Khaliel and Abou-Hailah, 1985). Ten AM fungi species were trapped from palm groves in southwest of Morocco (Bouamri et al., 2006). Fresh, young roots collected from around well-established date palm trees were heavily colonized by AM fungi (unpublished data at ICBA). AM fungi are important for plant growth especially in poor soil conditions (sand dunes, extreme sites, saline lands, polluted and degraded areas) where factors essential for plant growth are far below their optimum level. AM fungi occur naturally in saline environments in association with various host plants. This association is considered a survival mechanism both for the fungi and the plants through a bi-directional movement of nutrients (Gupta et al., 2000). Fungal hyphae are the most important agents in soil aggregate stabilization (Degens, 1997). Particle aggregation improves the soil physical properties that are required for better plant growth. Hyphae release a soil particle binding protein (glomalin) which has a positive correlation with the percentage of water-stable aggregates (Wright and Upadhyaya, 1998; Rillig et al., 2001, 2002).

AM fungi technology is the use of AM fungi and associated biota to enhance date palm establishment and growth at nursery and/or adult stages. This technology may be one of the low cost options to develop sustainable date palm production systems under saline/marginal quality soils and water conditions. Despite the significance of the mycorrhizae symbiosis with date palms, there are relatively few published reports on the interaction of AM fungi and date palms in UAE and/or the Arabian Peninsula and information on the use of AM fungi for date palm production under saline/marginal environments is either absent or scanty on a global view. The present experiment was designed to determine the potential use of AM fungi for the establishment and growth of date palms at various salinity and fertility levels under local conditions of the UAE.

MATERIALS AND METHODS

Experimental Site

The present experiment was carried out between March and September 2009 inside the plastic house facility of the International Centre for Biosaline Agriculture (ICBA (25°13'N;55°17'E)) Dubai, United Arab Emirates (UAE). The climate of the UAE is characterized by low rainfall, high evaporation rates and extreme temperatures in summer and winter months. Mean annual temperature varies between 3 and 48°C. Mean relative humidity in the country is 56.4%, with a maximum of 97% during summer in the coastal areas. Mean annual evaporation rate is 3350 mm for the whole country. The mean annual rainfall varies from 7 to 380 mm with an average of 130 mm (Brown, 2008). The soil texture is sandy (typic toripsamment, mixed hyperthermic). Biological activity in the soil is very low as they contain virtually no organic material. As a consequence, soils are highly deficient in nitrogen and are generally regarded as poor in terms of nutrient status.

Planting Materials and Methods

One-year-old in vitro seedlings of the date palm variety 'Khenizi' were obtained from The Ministry of Environment and Water (MoEW) of the UAE for the experiment. This variety was selected due to its importance in the local date palm production systems. Mycorrhizae inoculum BioMyc^{Vital} was obtained from a commercial company (BioMyc International, Germany) for mycorrhizal treatments. The inoculum consists, predominantly of hyphae and spores of arbuscular mycorrhizae (AM) fungi species *Glomus intraradices* (20 spores/ml or 200 propagules/ml) fixed on expanded clay particles of about 4 mm diameter.

Field soil was collected from the ICBA experimental farm and added into the organic material (OM) a mixture of peatmoss and perlite, obtained from the Emirates Bio-Fertilizer Factory (EBFF), UAE at 2:1 soil to OM ratio (v/v) and mixed thoroughly with a mechanical mixer. The mixture was devoid of nutrients at the beginning. The resultant substrate was filled into 20-L plastic containers and kept on the brick floor inside the plastic house. Seedlings were transplanted by making a 10×10 cm planting hole in the centre of the pot. For mycorrhizae treatments, 150 ml of BioMyc^{Vital} was placed at the bottom of the planting hole and covered with a thin layer of soil. Seedlings were then transplanted exactly at the top of the inoculum. It would ensure the quicker root colonization with mycorrhizae. As addition of BioMyc^{Vital} inoculum would modify the physical properties of the substrate, 150 ml of heat killed BioMyc^{Vital} inoculum was added for BioMyc^{Vital} control treatments. In addition, the inoculum was washed by passing through a 20-µm mesh screen and the resultant filtrate was added into the BioMyc^{Vital} control treatments to add the associated micro biota contained in the inoculum. Saline solutions of 5 and 15 dS m^{-1} were prepared by mixing fresh water (FW, EC 1-2 dS m^{-1}) with brackish water into the plastic tanks whereas, fresh water was used as a control to salinity treatment. The plants were irrigated daily with enough water that ensured free drainage to maintain the required salinity level in the root zone. For fertilizer treatments, two levels, one which is most commonly used by the commercial date palm growing nurseries for date palms establishment (called as full rate in this paper) and the other as 1/3 of the full rate (called as low rate) were applied. Plants were fertilized with full rate by adding 15, 30 and 5 g/month/plant of NPK (20:20:20), compost mix and Osmocote (12:13:13 + TE) respectively. For low rate, 1/3 of the full rate which is 5, 10 and 2 g/month/plant of the above mentioned fertilizers were added. In addition, standard cultural practices like insect control were followed throughout the whole course of the experiment.

Experimental Design, Data Recording and Analyses

The experiment was conducted using a modified split-split plot design with 6 replications of each treatment. Salinity level was the main plot and mycorrhizae and fertilizer were the sub and sub-sub plots respectively. The effectiveness of AM fungi was determined in terms of increased growth of mycorrhized over non-mycorrhized plants. Measurements were taken for plant height and trunk diameter at 180 days after transplanting. Plant height was recorded from the base of the trunk to the tip of the youngest leaf. Trunk diameter was measured just from above the base with a digital verneer calliper. Data were analysed with GenStat (Discovery addition 7) statistical software and significance of the treatment means was tested at ($p<5$) level of alpha.

RESULTS

Variation in growth parameters, trunk height and diameter were considered as the function of salinity, mycorrhizae and fertility treatments applied. Significance of means for plant height and trunk diameter is given in Table 1.

Plant Height

Analysis of variance confirmed the highly significant differences among means for plant height at all salinity, mycorrhizae and fertility levels. It was also clear that effect

of all the treatments was independent to each other because all interaction terms in ANOVA for salinity × mycorrhizae × fertility were non-significant. Plant height was declined with an increase in the salinity of irrigation water. Plants were the tallest with FW compared with high salinity (15 dS m^{-1}). Plant height of BioMyc^{Vital} plants was higher than non BioMyc^{Vital} plants at all salinity levels. Plant height of non-BioMyc^{Vital} plants varied from 21.8 to 18.3 cm whereas, plant height of BioMyc^{Vital} plants varied between 35.1 and 26.6 from FW to 15 dS m^{-1}. Height of low fertility plants was higher than plants with full fertilizer. Plant height of low fertility plants varied between 32.0 with FW and 24.7 with high salinity level. Whereas plant height of fully fertilized plants varied from 24.9 with FW to 20.3 with high salinity level. Plant height of BioMyc^{Vital} plants was higher than non-BioMyc^{Vital} plants at low and full rate of fertilizers. Plants with low fertilizer were taller than plants with full fertilizer rate. Plant height of BioMyc^{Vital} and non-BioMyc^{Vital} plants varied from 34.8 to 24.1 cm at low rate and 28.9 to 16.5 cm at full rate. Results for plant height are presented in Figures 1 to 3. Variations in plant height at various treatments are shown from Figures 8 to 10.

Trunk Diameter

It was confirmed by ANOVA that the effect of salinity on trunk diameter was non-significant but mycorrhiae and fertility means showed highly significant differences among them. No interaction was found among salinity, mycorrhizae and fertility treatments. Higher salinity lead to reduced trunk diameter. Trunk diameter of BioMyc^{Vital} plants was reduced from highest 29.7 mm to 24.3 mm and for non-BioMyc^{Vital} from 38.2 to 36.9 mm at FW and at high salinity (15 dS m^{-1}) respectively. The trunk diameter of fully fertilized was smaller than pants with low fertilizer rate. Trunk diameter of low fertility plants ranged from 40.1 mm to 33.7 mm and full fertility plants varied between 27.9 mm and 27.5 mm across all salinity levels. Trunk diameter of plants treated with BioMyc^{Vital} and low fertilizer was higher (42.7 mm) than plants with full fertilizer rate (36.1 mm). Whereas, trunk diameter of plants without BioMyc^{Vital} was 32.8 mm and 21.9 mm for low and full fertilizer rates respectively. Results of trunk diameter are presented from Figures 4 to 6.

DISCUSSION

Salinity limits plant growth by lowering osmotic potential in the soil solutions and ion toxicity. Our results showed that height and trunk diameter of date palm plants was reached to the highest with FW whereas both traits declined with increasing salinity level of the irrigation water. Although date palms can withstand long periods of drought coupled with high temperatures, they need a large quantity of water for vigorous growth, high yield and quality fruit (Chao and Krueger, 2007). Growth was decreased under saline conditions as lower osmotic potential caused by higher salinity made it difficult for the seedlings roots to absorb water whereas higher absorption of water was possible under low salinity or FW conditions. Seedlings inoculated with BioMyc^{Vital} had higher plant height and trunk diameter than BioMyc^{Vital} control plants regardless of salinity levels showing that mycorrhizal seedlings grow better than non-mycorrhizal seedlings under saline conditions. Plants show from weak to strong dependency on AM fungi (Janos, 2007). It has been suggested that benefits from mycorrhizae could be related to root system structure (Brundrett, 1991; Peat and Fitter, 1993). Species with lower root diameter and suberization, shorter lifespan, higher branching order and hair development would derive less benefit for mycorrhizal fungi than species with contrasting root traits (Brundrett, 1991). Date palms have limited (low densities of root hairs) root system which is highly colonized with mycorrhizae fungi under field conditions (pers. observaton) suggesting their strong dependency upon mycorrhizal symbiosis. The presence of AM fungi in date palms with high root colonization indicated that date palms highly benefit from the relationship with these fungi (Bouamri et al., 2006). Plants treated with BioMyc^{Vital} had a bigger root system than control plants (Fig. 7) which increased the water and nutrient absorption. The extensive network of AM fungal hyphae enormously

increased the rate of nutrient absorption even under saline/low osmotic conditions.

Growth of plants given low fertilizer rate was higher than plants fertilized with full fertilizer rate over all salinity and mycorrhizae levels. Growth of plants with low fertilizer rate, mycorrhized with BioMyc^{Vital} was higher that plants with full fertilizer either with or without BioMyc^{Vital}. These results confirm that AM fungi develop better under lower fertilizer regime levels than under high fertilizer input agriculture (see Sieverding, 1991 Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Hartmut Bremer Verlag, Friedland, Germany). Our results were in line with these findings as root growth of mycorrhized seedlings was higher than non-mycorrhized plants (Fig. 7). Mycorrhizae enhance the fertilizer use efficiency of plants as indicated in other studies. Palms are susceptible to nutrient deficiencies (Downer, 2004). A large amount of mineral fertilizers (NPK) is therefore added annually to enhance the establishment of young plantations and/or adult trees to attain high yields and good quality of dates (Barreveld, 1993). However, much of the N and P may not be taken up by plants and thus large amounts are leached down to the ground water. Inoculation with AM fungi enhanced the growth of date palm seedlings (Bouhired et al., 1992) and they absorbed a higher amount of P and K than non-inoculated seedlings (Al-Whaibi and Khaliel, 1994). Presence of mycorrhiza enhanced N, P and K in the desert soils (Al-Karaki et al., 2007). Phosphate content in both roots and shoots of date palm was significantly increased in the seedlings inoculated with AM fungi (Al-Karaki, unpublished data). The coefficient of P utilization in micropropagated oil palms increased 4-5-times after mycorrhization (Blal et al., 1990).

Plant height and trunk diameter of the seedlings fertilized with full fertilizer rate without or with BioMyc^{Vital} was lower than seedling with fertilized with low fertilizer rate. It confirmed that even mineral nutrients are available in abundance; date palms can not use them efficiently if AM fungi are not present. In the absence of AM fungi, micropropagated oil palms cannot use efficiently the phosphate fertilizer and inoculation increased fertilizer use efficiency by 2.7-5.6 times (Blal and Gianinazzi-Pearson, 1990). Uptake of nutrients, fruit growth and yields of date palms was increased when chemical and organic fertilizers were applied together (Bacha and Abo-Hassan, 1983). Organic fertilizers are generally compatible with mycorrhizae whereas, phosphorus-rich inorganic fertilizers inhibit the fungal growth (Amaya-Carpio et al., 2009). Mineral/inorganic P is readily taken up by plants while organic P is unavailable to the plants unless it has been mineralized or hydrolyzed into inorganic P. This conversion is mediated by phosphatase whose activity was greatly enhanced in the roots of mycorrhizal plants compared to non-mycorrhizal plants (Fries et al., 1998; Tawaraya and Saito, 1994). Although growth of seedlings with full fertilizer rate was increased in the presence of BioMyc^{Vital}, the growth of seedlings with low fertilizer rate with BioMyc^{Vital} was much higher. It is in line with the findings of Ezawa et al. (2000) who reported that high levels of P fertilization slowed down or inhibit initial mycorrhizae establishment and decrease mycorrhizal efficiency in plants.

CONCLUSIONS

The results generated at ICBA about the efficiency of mycorrhizal technology for production of date palm clearly indicate that high quality mycorrhizal inocula have a real potential to improve date palm productivity under the conditions of saline soils in the UAE and the Arabian Peninsula. The results also showed that high chemical inputs like fertilizers and pesticides are not necessary for optimum date palm growth. It appears that biological production methods are superior. There is a clear rationale for mycorrhizal technology use in nurseries. The innovative technique of introducing mycorrhizal fungi to plants during the propagation and planting phases of their lives might reverse the problems of high chemical input agriculture to more natural biological production systems resulting in improved growth rates, plant establishment and more consistent and stable yields with complementary reductions in water and fertilizer use (Sieverding, 2008). This is especially important with tissue cultured date palms due to the great benefits of enhancing the health, survivorship and establishment when planted under

stressed or poor environmental conditions. It is coupled with the enormous potential reduction in the overall costs due to avoiding high mortality of tissue cultured plants in addition to complementary reductions in fertilizer and water use.

ACKNOWLEDGEMENTS

The International Centre for Biosaline Agriculture (ICBA) provided full support to carry out the present study. The Ministry of Environment and Water (MoEW) provided in vitro date palm seedlings. BioMyc International supplied the mycorrhizae inoculum (BioMyc^{Vital}). Emirates Bio-Fertilizer Factory, UAE supplied the organic matter. We appreciate Eng. Basel for the continuous supply of saline solutions. Non-stop scientific and technical guidance of Dr. Ahmed Al-Masoom, Dr. Ewald Sieverding, Dr. Hassan Shabana, Dr. Mohammad Badawi and Dr. Ghazi Al-Karaki is highly appreciated. Continuous encouragement and help of conservationist Volkmar Albers in all aspects had been the key to complete this experiment.

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Tables

Table. 1. Mean squares of plant height and trunk diameter of variety "Khenizi" after 180 days of transplanting.

Source of variation	df	Mean square	
		Plant height (cm)	Trunk diameter (mm)
Salinity	2	242.8**	4.08^{NS}
Mycorrhizae	1	2392.0**	66.4**
Salinity × mycorrhizae	2	48.4^{NS}	1.6^{NS}
Fertility	1	820.1**	42.9**
Salinity × fertility	2	28.7^{NS}	1.8^{NS}
Mycorrhizae × fertility	1	13.4^{NS}	2.5^{NS}
Salinity × mycorrhizae × fertility	2	22.4^{NS}	0.95^{NS}

**=highly significant (p<5).
NS=non significant.

Figures

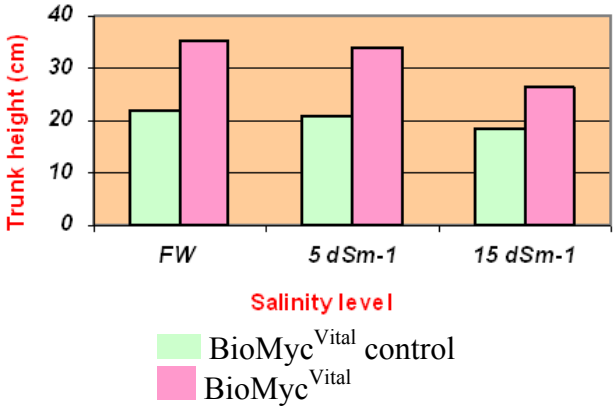

Fig. 1. Plant height of variety 'Khenizi' with BioMyc^{Vital} control and BioMyc^{Vital} at three salinity levels.

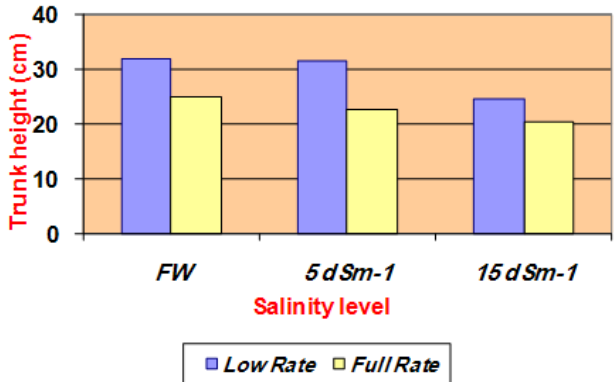

Fig. 2. Plant height of variety 'Khenizi' with low and full fertilizer rate at three salinity levels.

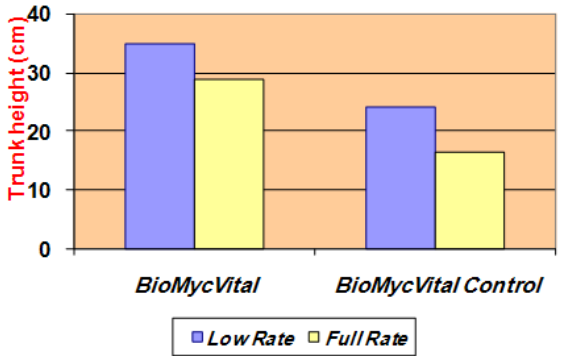

Fig. 3. Plant height of variety 'Khenizi' with low and full fertilizer rate with BioMyc^{Vital} and BioMyc^{Vital} control.

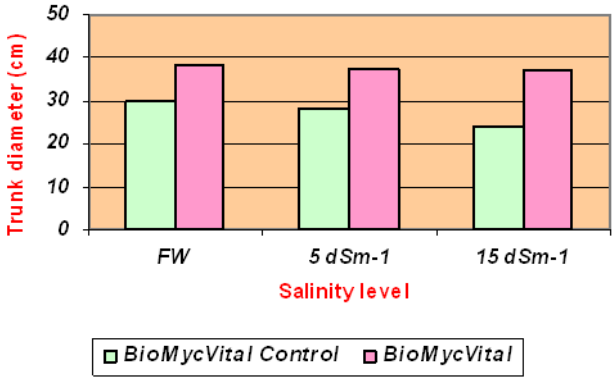

Fig. 4. Trunk diameter of variety 'Khenizi' with BioMyc^{Vital} control and BioMyc^{Vital} at three salinity levels.

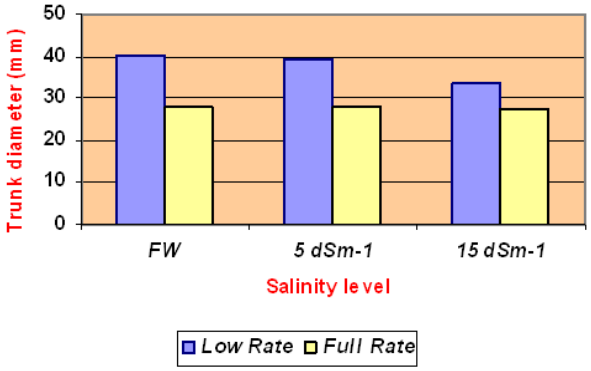

Fig. 5. Trunk diameter of variety 'Khenizi' with low and full fertilizer rate ath three salinity levels.

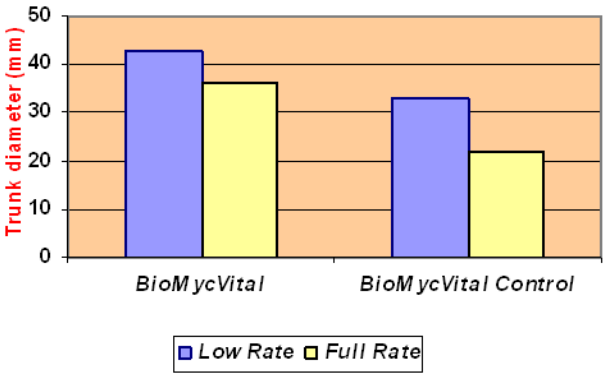

Fig. 6. Trunk diameter of variety 'Khenizi' with low and full fertilizer rate with BioMyc^{Vital} and VioMyc^{Vital} control.

Fig. 7. Root growth of date palm seedlings (left) without BioMyc^{Vital} and (right) with BioMyc^{Vital} after 180 days of transplanting.

Fig. 8. Plants with BioMyc^{Vital} (left) with low fertilizer rate (right) with full fertilizer growing with fresh water after 180 days of transplanting.

Fig. 9. Plants with BioMyc^{Vital} (left) with low fertilizer (right) with full fertilizer growing at 5 dS m^{-1} after 180 days of transplanting.

Fig. 10. Plants with low fertilizer rate (left) without BioMyc^{Vital} (right) with BioMyc^{Vital} growing at 15 dS m^{-1} after 180 days of transplanting.

Development of a New Date Palm Pollinator

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Keywords: *Phoenix dactylifera* L., mechanization, peripheral dispersion, pollen dispersion, fruit set, pollen dilution

Abstract

Commercial date production necessitates artificial pollination that ensures good fruit set and overcomes disadvantages of dichogamy and also reduces the number of male palms in a planting area. The traditional method of artificial pollination is based on the climbing of the palms which is a tedious and costly operation and needs much time to complete. Current mechanical pollinators eliminate the need to climb the palms and thus reduce the work intensity and time, but they suffer from low reliability and controllability due to their design and need two workers to do the operation. A new electrical apparatus for pollinating date palms has been designed and developed in this research. The dispersing system used is of peripheral dispersion method and is completely different from the previous designs. Thus the pollination feasibility and controllability have been enhanced while the operation time and tool size, weight, and cost diminished in the new tool in comparison with current mechanical pollinators. The preliminary evaluation of the tool performance on the 'Barhee' cultivar in Ahwaz region showed that though it lead to more fruit set than traditional and mechanical methods, the difference was not significant and thus the tool succeeds in obtaining the functional goal besides the aforementioned advantages. Mean fruit set attained by the developed tool, mechanical pollinator and traditional method was 68.12, 62.04 and 64.94% respectively. This tool handles 200 cubic centimeters of pollen mixture by which about 120 palms (about 1 ha date palm plantation) could be pollinated in each charge by only one worker.

INTRODUCTION

Pollination is one of ten principal date palm cultivation practices. This operation has a great role in fruit development and thus it is the most important operation which is done annually. The traditional pollination method involves the insertion of 3-5 strands of male spadix upside down into the female spadix (Eeta, 1986). It is an intensive, tedious, and very expensive operation and is not suitable for commercial production of dates. Many research works have been done in order to optimize this operation through using mechanical devices. Investigations of previous works have proved that the mechanical pollination results in less operation time and cost in comparison with the traditional method (Eeta, 1986, 1988) and thereby improves the land usage through reducing the number of male palms needed as a pollen resource (Albozahar, 2003). Some mechanical pollinating devices have been developed by Alexander (1952), Yost (1957), Loghavi (1993), Haffar (1999), Al-Rawi (2001) and Obaidi (2001). Unfortunately, the proper devices have not developed till now and the operation is mostly done traditionally in Iran. Problems related to both design and performance of some developed devices are the major reasons for this problem. Heaviness, existence of air tank on the back of the worker, and mostly jamming of the pollinator tubes by pollen mixture are major problems (author observations). Thus, this research has been done to eliminate the aforementioned problems and to facilitate the operation through presenting the novel design.

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MATERIALS AND METHODS

This research was conducted with the aim to develop an appropriate pollinating apparatus for dispersing pollen mixture on date palm spadixes in three phases. In the first phase design inputs including design requirements and pollen attributes were determined and an overall design was selected from possible ones. The detailed design becomes ready at the end of this stage. The parts were developed and the apparatus was assembled and became ready for testing in the second phase. Performance of the developed apparatus in pollinating date palms was evaluated in the third phase.

In the third phase the cultivar 'Barhee', a commercial date cultivar in Khuzestan province of Iran, was selected for this experiment during the 2008 season. Nine medium height palms (5-6 m) in the Ahwaz date palm germplasm collection were randomly selected in a Grouped Balanced Block Design with 3 replicates. Each replication consisted of two bunches. The pollination methods used were: 1) involving the insertion of 3 male strands of male spadix upside down into the female spadix, 2) a mechanical pollen duster consisting of an ordinary steel tank chemical applicator as air supplier to push pollen through a pollen mixture container mounted on an aluminum tube by compression and 3) the developed electrical pollen duster. The same pollen of the 'Kashani' cultivar was used for the entire experiment and all pollinated spadixes were covered by specially designed bags with a plastic window just after pollination. Pollen for the mechanical and electrical pollinators was diluted through mixing with wheat flour (grade 1) 3 levels of 10% (group A), 20% (group B), and 30% (group C) of pure pollen in the pollen mixture (100×g/g). The mixture of pollen and flour was released 2 times on each spadix; after the spadix opens and 2 days after that to ensure fruit set and uniformity.

Fruit samples were collected to represent 'kimri' stage: hard, elongated, dark green fruits collected 10 weeks after pollination. Five strands from each bunch were taken randomly as a sample and developed fruits, drop fruits, and unpolinated fruits were counted on these strands. Fruit set was calculated by dividing developed fruits by total fruit sites multiplying by 100.

RESULTS AND DISCUSSION

Description of the Developed Tool

Figure 1 represents the developed device sketch. This device consists of an extending pole (1), dispenser (2), pollen pipe (3), nozzle (4) and remote controller (5). The pole is made of aluminum pipes, 1.5 m length steeply increasing in diameter from top to bottom. The main objective of this part is to bring the dispenser close to the spadixes without the need to climb the palm.

The dispenser consists of a double-wall pollen tank, blower, and top cap. The pollen tank has a cylindrical body, an adjustable pollen tank, and an air distributor. The adjustable pollen tank is fixed inside the cylindrical body right above the air distributor. The pollen tube is small in diameter which is extended from the top cap and is bent in 45 degrees to make a targeted pollination. Some types of nozzles could be used to enhance the pollination process.

The dispensing operation is controlled remotely by a control set. In general a pair of transmitters in the control set and receiver in the dispenser equipped with adjustable timer is used. When the timer is set on by the worker through a button on the controller, the blower is set on to a pre-adjusted time and then it is turned off automatically. Figure 1 also shows the device in operation in a date palm orchard.

RESULTS OF THE PERFORMANCE EVALUATION

Variance analysis showed no significant difference in fruit set means between treatment groups and evaluated pollination methods within groups (Table 1). The mean fruit set for traditional, mechanical and electrical methods was 64.94, 62.04 and 68.12% respectively (Fig. 2). It seems that the developed device gained more fruit set than traditional and mechanical ones, although the difference is not significant. Based on the

results the device gained the main goal in successfully pollinating dates in comparison with the traditional method which always gives better results because of the existence of permanent and accessible pollen for spadixes in this method. On the other hand, the developed device offers the farmer more maneuverability, feasibility, while the weight, power, and cost decrease. Samples of date palm strands pollinated by three methods are shown in Figure 3. Maximum fruit set gained by traditional, mechanical, and electrical methods were 78.99, 72.79 and 82.84% respectively. Mean fruit set for different pollen dilution levels are shown in Figure 4. Based on the results the 1st level of 10% pollen per pollen mixture could be recommended with regard to restricted pollen resources and cost, but detailed economical appraisal of less and more dilution levels must be done to recommend the economic one.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results the newly developed device is capable of successfully pollinating date palm spadixes. The new device offers unique and superior features both in performance and cost in comparison with traditional and mechanical methods that make the device more acceptable, thus it could be offered to low scale farmers. The pollination with this device could be done by high pollen dilution levels to decrease the operation cost. The pollen dispersing mechanism in the developed device allows higher and lower dilution levels while maintaining the uniformity. Thus, these levels should be evaluated to optimize the pollinator. In overall the new device is recommended for low scale date palm farmers while precise study of the new device from both time and cost aspects, detailed performance analysis of the device suitability for other commercial varieties, and evaluation of pure pollen pollinating by this device are also recommended. This device is patented in Iran's patent office under no. 43620.

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Tables

Table 1. Analysis of variance for fruit set data.

Source	DF	SS	MS	F
Replication	2	7184.722	3592.361	--
Treatment group	2	4648.167	2324.083	2.176051^{ns}
Between groups error	4	4272.111	1068.028	
Treatments within group A	2	111.5	55.75	0.117375^{ns}
Treatments within group B	2	518	259	0.545295^{ns}
Treatments within group C	2	903.5	451.75	0.951108^{ns}
Within groups error	12	5699.67	474.9722	
Total	26	23337.67		

ns: not significant

Figures

Fig. 1. Sketch of developed electrical pollinator (left) and the pollinator in work (right).

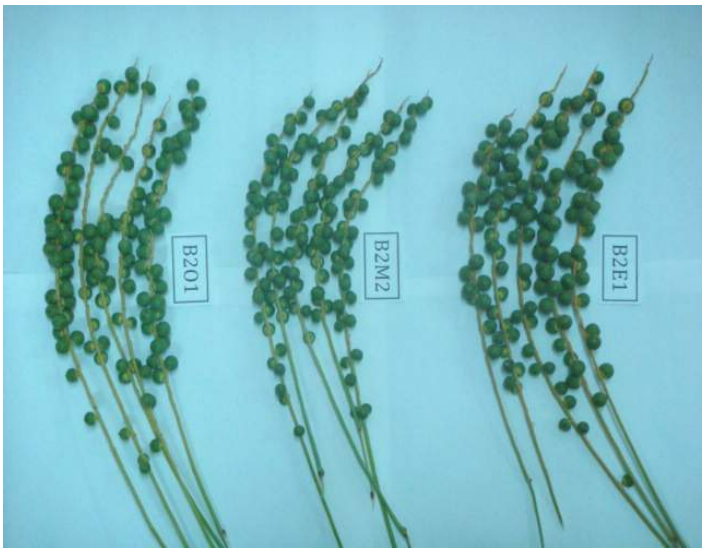

Fig. 2. Samples of pollinated date palm strands in kimri stage. From left to right: traditional, mechanical, and electrical pollinating.

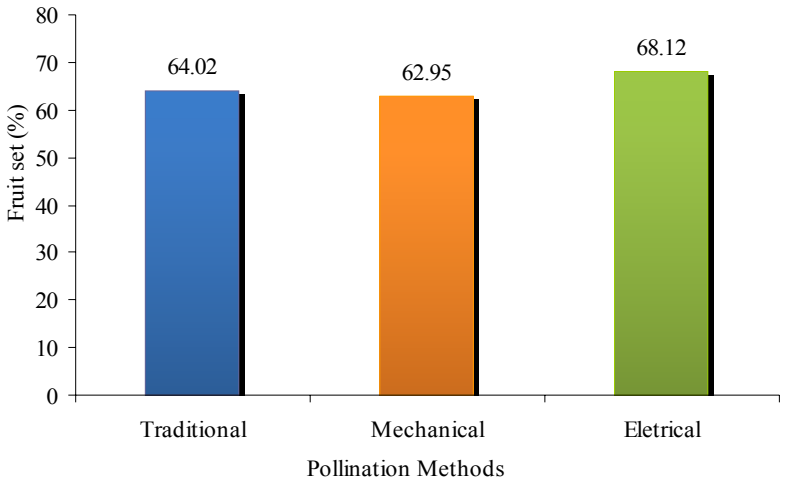

Fig. 3. Mean fruit set gained by the methods.

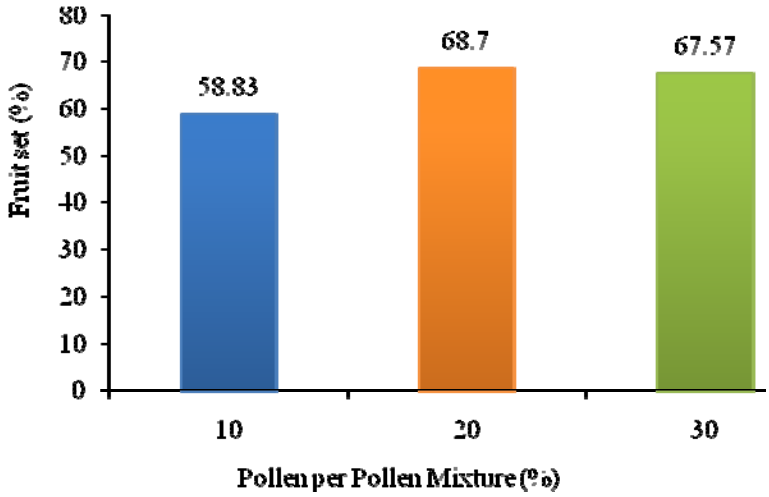

Fig. 4. Effects of pollen dilution on fruit set.

Phenolic Compounds as Markers for Algerian Cultivars of the Date Palm *Phoenix dactylifera* L.

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Keywords: palm tree, flavonoid content, HPLC profiles, conformity

Abstract

The objective of this research was to make an inventory of the cultivars of the *Phoenix dactylifera* L. by searching biochemical-markers. Fifty individuals which belong to 9 cultivated palm trees were analysed for their flavonoid content. The description of HPLC profiles of glycosyl flavones and glycosyl flavonols initially enabled us in the first time to identify 15 compounds. The structural analysis showed that flavonic aglycones are specific markers to the date palm. The second time we established that the diversity of these flavonic glycosides, considered as markers, permits to distinguish two sets of cultivated date palm: some are well homogenous ('Ahartane', 'Aghamu') others not ('Deglet nour', 'Takerbucht'). The second set corresponds to cultivars submitted to intensive cultivation and phenotypical selection by date palm farmers. We show a negative correlation between the content of flavones and the degree of lignification in the date palm which suggests that the process of lignification is more rapid in resistant cultivars; we can say that resistance to Bayoud is constitutive. Moreover, we made a test of conformity between the adults and vitro-plants of 'Feggous' using the flavonic content. A second test was conducted between male and female plants of two cultivars, 'Deglet Nour' and 'Degla Beida', using the same compounds. We revealed that flavonoids cannot constitute chemical markers related to the sex; there is conformity between the male and female palm trees of the same cultivar.

INTRODUCTION

Because of its food utility and its socio-economic importance, the date palm *Phoenix dactylifera* L. remains a much appreciated fruit-bearing tree in the pre-Saharian area. Starting from the characters of the fruit and the seed, the description of certain number of forms has led to the recognition of cultivars, an empirical step which can be regarded as an approach of genetic evaluation. However, in this context, date palm farmers have often selected, crossed and specialized the representatives of this dioic species, resulting in a phenotypical diversity which makes this knowledge problematic. The more so as a certain number of characters are not stable and depend on the phenological stage. The recognition of biochemical markers whose synthesis is under genetic dependence, permits to identify the palm tree at any age and any season, because polyphenolic polymorphism is an original molecular reflection of genetic diversity.

MATERIALS AND METHODS

Vegetative Material

The study is related to 50 individuals (adult female seedlings) of date palm accounting for 9 cultivars obtained from the INRA (National Institute for Agronomic Research) experimental station at Adrar (Algerian Sahara). The palm leaflets (external palms) were cleaned then dried in free air and protected from light. This plant material was finely ground for biochemical analysis.

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Preparation of Extracts

Two grams of vegetative powder were submitted during 20 min to hot hydro-alcoholic extraction (H_2O/EtOH: 7/3) in presence of glass balls. The extract is filtered, dry evaporated under vacuum, then dissolved in boiling water. From the filtered solution, heterosidic compounds are extracted by n-butanol; the dry residue is taken again by methanol for chromatographic analysis.

Chromatographic Analysis

Liquid chromatography high pressure analysis (HPLC) was carried out on a Kontron apparatus equipped with a nucleosil C18 column, 5 microns, 250 mm. The elution system is a gradient of acetonitrile in water with 20% of acetic acid. Spectrophotometric detection is carried out at the wavelength absorption 328 nm corresponding to the flavones glycosides. This system detects 15 peaks, each chromatographic information being able to correspond to a molecule.

Identification of Flavonoid Compounds

Flavonic compounds are identified in HPLC by their retention times and UV-spectra compared to witnesses. Confirmation is obtained by TLC (Thin Layer Chromatography) with determination of the Rf of the spots and their fluorescence under UV light, with or without revealing reagents of polyphenols. The identification is supplemented by acid hydrolysis (HCl 2 N, 100°C, 40 mn) making it possible to reach the aglycones (UV spectra) and the corresponding sugars (TLC) mutually engaged in the O-glycosidic bonds.

Quantitative Determination of Flavonoids.

Flavonoids are quantified by the height of their HPLC peaks and expressed as a percentage by dividing the height of each peak by the sum heights of all the detected peaks. This relative weighting makes it possible to disregard total quantity per individual, which can vary according to environment and sampling.

RESULTS AND DISCUSSION

Flavonic Aglycones

Our chemotaxonomic studies previously showed that flavonic aglycones (flavones, flavonols and anthocyanin) are specific markers of the date palm (Ouafi et al., 1988), (Figs. 1 and 2).

Among all individuals studied we found: as anthocyanin: cyanidin (CY), Flavonols - flavones: two flavonols (quercetin (Q) - isorhamnetin (Q)) and three flavones (Luteolin (L) - Tricine (T) - Chrysoeriol (CHR)).

As we showed, based on levels of each compound, the weight of discriminating variables (aglycones flavonic) is limited (Fig. 5).

Flavonic Heterosidic

Our analysis relates to the diversity of flavonic heterosidic class. The 9 cultivars of the INRA date palms collection can be distinguished by their flavonic chemotypes, founded on 15 molecular forms (Fig. 3).

Qualitative Analysis

The results of the structural analysis of the different cultivars from *Phoenix dactylifera* L. show a great diversity in the expressed molecular forms (Table 1). These are fifteen, founded on two aglycones of flavonols (quercetin, and its 3-O-methylated derivative: isorhamnetin), and three flavone aglycones (luteolin, its 3'-O-methylated derivative: chrysoeriol, and tricine, a 3', 5'-O-dimethylated flavone). Eight of these 15 molecules (D, H, J, L, M, N, Q, and R) are specific because they are present in all the cultivars, generally in high content. In order to eliminate their "background noise", we

restricted our preliminary qualitative analysis to the 7 other (minor) flavonoids (Table 2). This first approach of the infraspecific flavonoids distribution lets 3 subsets appear, with a first dichotomy based on the presence of flavonols (compound K = isorhamnetin-3 O-galactoside), a second on the presence of flavones (compound S = homo-orientin pentoside). One will note the extreme structures of these two markers, and the polyphenolic poverty of the cultivars 'Ahartane' and 'Aghamu', particularly as opposed to the richness of 'Tazarzait' and 'Degla Beida'.

We note the presence of the compound L '(isorhamnetin - 3-0-glucoside) only in the cultivar 'Deglet Nour'. We are tempted to say that this flavonol glycoside may be a marker of this cultivar.

The results tend to designate the most hydroxylated compounds such as anabolites initial preferences. The reports of specific activities within each flavonic group show little variation around the unit depending on the duration of the biosynthesis, suggesting instead affiliations "star" at different speeds, from intermediate precursors of each chemical family. The application regarding the *Phoenix dactylifera* L. whose original character was revealed, leads us to hypothesize about the ancestral character of 'Deglet Nour' (the least rich in glycosides substituted) compared the rest of the cultivars studied in this work.

Structural Analysis of Phenolic Acids

The phenolic acids were obtained after acid extraction with HCl 2 N. Structural analysis of compounds in the extracts was done using chromatographic and spectrophotometric techniques. The study of the HPLC profiles of leaflets of palm trees showed a total of 10 peaks (Fig. 4).

The presence in the *Phoenix dactylifera* L. of p-hydroxybenzoic vanillic and syringic acid, which are precursors of lignin, indicates that it is a species that has lignin. We found that the studied compounds are present in the HPLC profiles of all individuals belonging to the nine cultivars. Thus they may be markers of cultivar. They characterize the species *Dactylifera*. Finally, the results of structural analysis of phenolic acids show that we find in the leaflets phenolic acids parietal reported in roots of date palm (Ziouti et al., 1998; El Modafar et al., 1998) p-hydroxybenzoic acid, p-coumaric, ferulic acid and sinapic acid.

The quantitative aspect could be used in the intercultivar conduct of date palm towards bayoud. Thus, the resistant genotypes accumulate higher quantities of phenolic acids in their roots (Ziouti, 1990). This test could serve as a biochemical marker of the date palm resistance to *Fusarium oxysporum* fsp. *albedinis*. These phenolic compounds were often used in relation with plant pathology in several plant species (Harborne, 1989; Misaghi, 1982). Especially, as some are precursors of lignin. The merits of our work show a negative correlation between the content of flavones and the degree of lignification in the date palm suggesting that the process of lignification is more rapid in resistant cultivars; we can say that resistance is constitutive.

It should be noted that these results derive from chemical analyses of phenolic compounds carried out on the leaflets of adult female palm trees.

Test of Conformity

We carried out a test of conformity between the flavonic content of date palm leaflets and that of seedlings resulting from in vitro cultures of the same cultivar. The qualitative analysis of flavonoids shows the presence of the same compounds in the cultivars 'Feggous' and 'BouFeggous': 1 anthocyanin: cyanidin; 2 flavonols: quercetin and isorhamnetin; 3 flavones: luteolin, and the tricine chrysoeriol; 12 compounds heteroside.

These results allow us to suggest that qualitatively both 'BouFeggous' and 'Feggous' have the same flavones profile and chemically morphological results seem to confirm that the (Benkhalifa, 1989) Algerian 'Feggous' and the Moroccan 'BouFeggous' are two names for the same cultivar. This similarity is interesting since in vitro cultures

open a perspective to large production of a cultivar selected for its qualities. Our work constitutes a positive test of conformity between mature plants and in vitro seedlings.

Flavonoids cannot constitute chemical markers related to the sex as there is conformity between the female and male palm trees of the same cultivar.

CONCLUSIONS

The study of *Phoenix dactylifera* L. under different aspects showed that the species is very interesting because of the originality of its secondary metabolites responsible for an important biochemical polymorphism. The foliage is rich with polyphenol since four flavonic families: proanthocyanidins, flavones, flavonols these two last in form O and/or C - glycosylated were found.

This originality is reflected by the fact that the aglycone forms are specific markers while heteroside native forms are markers of the cultivar, phenolic acids are related with resistance to the *Fusarium oxysporum* fsp *albedinis*.

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Tables

Table 1. Structure of the 15 flavonic glycosides identified in the date palm.

D: Orientine (Lutéoline 8-C-glucoside) C-Glucosyl-8 lutéoline	H: Homo-orientine (Lutéoline 6-C-Glucoside) C-Glucosyl-6 lutéoline
J: Quercetin 3-galactoside Galactosyl-3 quercetin	K: Isorhamnétine 3-galactoside Galactosyl-3 isorhamnétine
L' : Isorhamnétine 3-glucoside Glucosyl-3 isorhamnétine	L: Quercetin 3-rhamnoglucoside (rutin) Rhamnoglucosyl-3 quercetin
M: Lutéoline 7-glucoside Glucosyl-7 lutéoline	N: Lutéoline 3-rhamnoglucoside Rhamnoglucosyl-7 lutéoline
O: Chrysoériol 7-rhamnoglucoside Rhamnoglucosyl-7 chrysoériol	P: Tricine 7-rhamnoglucoside Rhamnoglucosyl-7 tricine
Q: Chrysoériol 7-glucoside Glucosyl-7 chrysoériol	R: Tricine 7-glucoside Glucosyl-7 tricine
S: Homo-orientine X-O-pentosid O-Pentosyl-x homo-orientine	T: Homo-orientine x-O-hexoside O-Hexosyl-x homo-orientine
V: Chrysoériol X-C-glucoside C-Glucosyl-x chrysoériol	

Table 2. Restricted flavonic profile of the 9 cultivars of date palm.

Flavonols			Flavones				
Heterosides (Aglycone)	K Irh	L' Irh	P Tri	Q Chr	V Chr	S Lut	T Lut
Cultivars							
Subset A (presence of K = flavonol, presence of S + T = lutéoline)							
Degla Beida	+	-	+	+	+	+	+
Ghars	+	-	+	-	+	+	+
Tazarzeit	+	-	+	+	+	+	+
Deglet Nour	+	+	-	-	+	+	+
Tinaceur	+	-	+	-	-	+	+
Subassembly B1 (absence of flavonols, presence of S + T = lutéoline)							
Takerbucht	-	-	-	+	+	+	+
Tilemsu	-	-	+	-	+	+	+
Subassembly B2 (absence of flavonols, absence of S)							
Ahartane	-	-	-	-	-	-	+
Aghamu	-	-	-	+	-	-	-

Figures

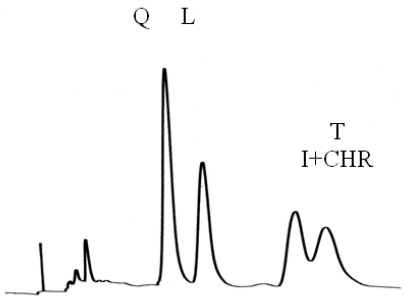

Fig. 1. HPLC profile of flavonic aglycones.

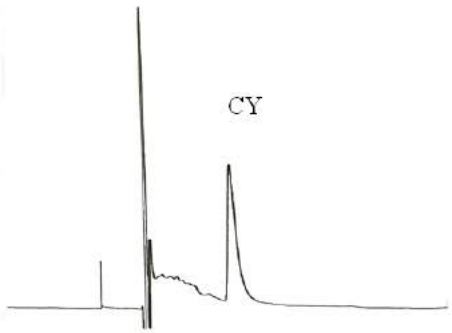

Fig. 2. HPLC profile of anthocyanin.

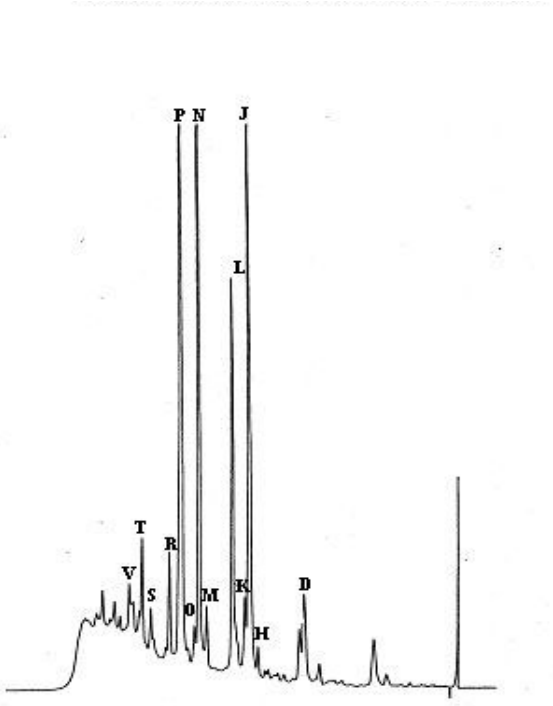

Fig. 3. Flavonic profile flavonic of 'Degla Beida'.

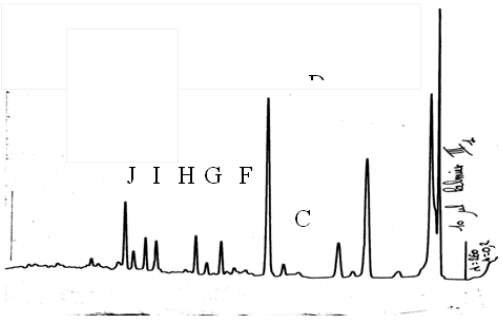

Fig. 4. HPLC profile of phenolic acids from leaflets of date palm.

A: catéchol
B: protocatéchic acid
C: gentisic acid
D: arahydroxybenzoïc
E: vanillic acid
F: caffeic acid
G: syringic acid
H: p.coumaric
I: ferrulic acid
J: sinapic acid

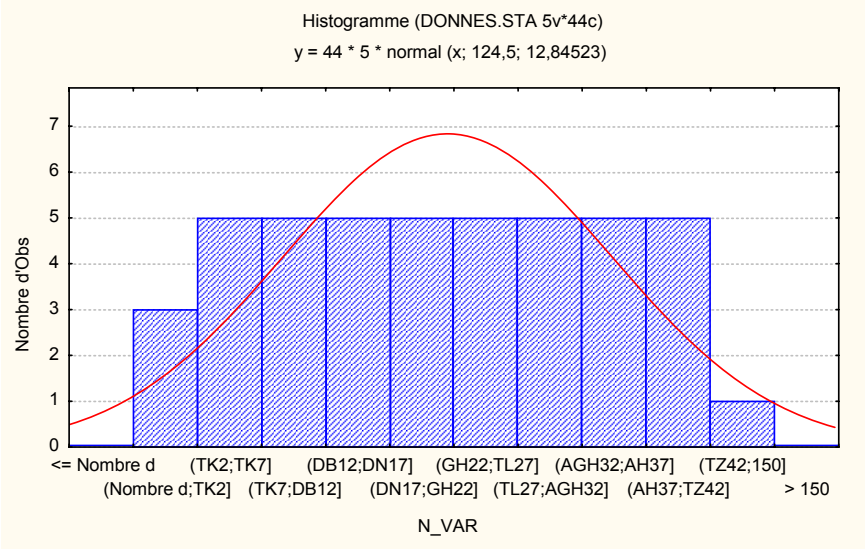

Fig. 5. Histogram.

Effect of Pollen Grains Suspensions Spraying on Yield and Fruit Quality of 'Saidy' Date Palm

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Abstract

The effect of spraying pollen grains suspensions as a pollination method of 'Saidy' date palms which combines both mechanical pollination and fruit thinning was studied during the 2005, 2006 and 2007 seasons. Pollination was achieved by spraying the pollen grain suspensions at 0.62, 1.25, 2.50, 5.00, 10.00 and 20 g pollen plus 1 g ascorbic acid/L.

The obtained results indicated that there is a significant reduction on fruit set and bunch weight as the pollen grains suspension concentration is reduced. This reduction occurs gradually with decreasing the pollen grains suspension concentration from 20-0.62 g/L. On the other hand, an improvement in fruit quality was observed with the reduction of the pollen grains suspension concentration used. Such improvement occurs with decreasing the used pollen grains suspension concentration from 20-0.62 g + 1 g ascorbic acid/L.

It could be concluded that using a suspension containing 2.5-5.0 g of pollen grains and 1 g ascorbic acid/L water would lead to a considerable yield with good fruit quality, in addition to the improvement of the efficiency of the pollination process.

INTRODUCTION

Artificial pollination is considered the only way for commercial date production. Hand pollination in date palm is necessary for successful fruit set and fruiting (Nixon, 1951; Ream and Furr, 1970).

Pollen grain germination is closely related with both the environmental conditions and stigma respectively. High relative humidity was shown to damage date pollen grain, whereas, high temperature induces poor germination of pollen grains. Pollination of 60-80% of the female flowers is considered satisfactory and will usually lead to a good fruit set. The pollination efficiency is affected by several factors and consequently fruit set is highly dependent on these factors. The pollination time, flowering period of male palm, the type of pollen, its viability and amount and the female flowers receptivity are the main factors to take into account (Brown and Perkins, 1969; Brown et al., 1984; El-Salhy et al., 1997).

The success of fruit production depends mainly on the success of pollination. The mechanical pollination had a positive effect on the total yield, due to increasing fruit set. It seems to be a viable solution to economize the quantity of pollen and improve the time management of the pollination especially for precocious, later and shorter receptivity cultivars (Haffer et al., 1997; Al-Awsaibi et al., 2007).

The mechanical pollination requires mixing the pollen grains with a bulky material to minimize the amount needed of pollen grains. This bulky material must be available, cheap, dry and with specific gravity close to that of the pollen grains in order to obtain an homogeneous mixture. They could be wheat flour, wheat bran, and crushed dry male flowers after the pollen grains extraction (Mostafa, 1994; Ahmed et al., 1995; El-Makhtoun and Abdel-Aal, 1995; Shabana et al., 1998).

Mixing pollen grains with various carriers and nutrient minerals were beneficial in establishing mechanical pollination and obtaining an economical yield with good fruit quality. Also, it is responsible for enhancing pollination efficiency (Furr and Hewitt, 1964; Khalil and Al-Shawaan, 1982; El-Kassas and Mahmoud, 1986; El-Mardi et al.,

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1995; Hussein and Hassan, 2001; Ragab et al., 2004; Ashour et al., 2004; El-Salhy et al., 2007).

Moreover, using pollen grains mixed with pure water was successful in pollination of 'Fard' date palm and it was recommended to use 0.5g/L of water for its pollination (Al-Sabahi et al., 2006).

Also, it was recommended to use 0.5 g of pollen grains/L of water concentration to pollinate 'Jabree' and 0.1 g/L for 'Helaly Oman' (Alabri et al., 2006).

So, this study aimed to innovate an untraditional method in date palm pollination which combined both mechanical pollination and fruit thinning in addition to get high yield with good quality.

MATERIALS AND METHODS

This study was conducted in the date palm Research Farm in the Agricultural Research Station, at El-Kharga Oasis, New Valley Governorate, Egypt, during three successive growing seasons 2005, 2006 and 2007, on 35-years-old 'Saidy' date palm cultivar (as semi dry date palm cultivar).

Eight date palms that are uniform in vigour and in good physical condition, free of insect damage and diseases were selected. The number of spathes per palm was adjusted to ten by removing excess earliest, latest and smallest clusters for achieving the following eight treatments:

1. Hand pollination by inserting 7-10 strands/bunch.
2. Spraying pollen grains suspension (20 g pollens/L water).
3. Spraying pollen grains suspension (20 g pollens + 1.0 g ascorbic acid/L water).
4. Spraying pollen grains suspension (10 g pollens + 1.0 g ascorbic acid/L water).
5. Spraying pollen grains suspension (5 g pollens + 1.0 g ascorbic acid/L water).
6. Spraying pollen grains suspension (2.5 g pollens + 1.0 g ascorbic acid/L water).
7. Spraying pollen grains suspension (1.25 g pollens + 1.0 g ascorbic acid/L water).
8. Spraying pollen grains suspension (0.62 g pollens + 1.0 g ascorbic acid/L water).

These treatments were applied on the same palm. Pollination was uniformed in respect of source and method to avoid residues of metaxenia. The experiment was set up in a complete randomized block design with eight replications of one bunch each.

Hand pollination as well as pollination treatment sprays were applied at the third day of spathe cracking. Sprays of pollen suspension are thoroughly applied to the bunch by small hand sprayer (1/2 L capacity) at the amount of 50 ml/bunch. To prevent contamination of pollens, after the spraying of pollen suspension, every bunch was bagged by paper bags which were removed after four weeks.

Data concerning the temperature (°C) and relative humidity (%) during the pollination periods of the present study are given in Table 1. They were obtained from the El-Kharga Meteorological Station.

Measurements

1. Fruit set %. Fruit set percentage was evaluated after one month of pollination. Five female strands per bunch were randomly selected from each replication. The number of fruit set was recorded, then fruit set percentage was calculated as the following equation:

$$\text{Fruit set \%} = \frac{\text{Number of fruits setting on the strand}}{\text{Total number of flowers per the strand}} \times 100$$

2. Yield and Fruit Quality. Bunches were harvested at tamr stage (last week of September), fruit weight/bunch (kg) was recorded. 25 fruits from each bunch were picked at random for determination of the following physical and chemical fruit characters:

1. Fruit and seed weight (in g), then pulp percentage was calculated.
2. Fruit length (L) and diameter (D) were measured by vernier caliper (in cm).
3. Percentages of total soluble solids by hand refractometer.
4. Percentage of total, reducing and non-reducing sugars by using the volumetric method that was outlined in A.O.A.C. (1985) by Lane and Eynon.

All the obtained data were tabulated and subjected to the proper statistical analysis of variance using the LSD test for recognizing the significance differences among the various treatment means according to the method outlined by Snedecor and Cochran (1980) and Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Yield Index

Fruit weight/bunch is an indicator for the yield of palm trees since the number of bunches on the palm was constant.

Data illustrated in Table 2 show the effect of spraying the pollen grains suspension either with or without ascorbic acid at different concentrations on fruit set percentage and fruit weight/bunch of 'Saidy' date palm during the 2005, 2006 and 2007 seasons.

Data show that there are insignificant differences in fruit set percentage and fruit weight/bunch due to pollination by using either pollen grains suspension (20 g/L) alone (T_2) or (20 g/L) plus 1 g ascorbic acid (T_3) compared with the traditional hand pollination (T_1). The fruit set percentage values were 74.74, 74.96 and 74.67% whereas, the fruit weight/bunch were 9.66, 10.02 and 9.61 kg as an av. the three studied seasons due to pollination by using suspension of pollen grains either 20 g/L alone (T_2) or 20 g/L plus 1 g ascorbic acid (T_3) and traditional hand pollination, control (T_1), respectively.

However, there was a reduction on the fruit set percentage and fruit weight/bunch with reducing of the pollen grains suspension concentration. The reduction of these traits was associated with decreasing the pollen grains suspension concentration from 20 g/L (T_3) to 0.62 g/L (T_8). Moreover, there was a significant decrease in bunch weight due to pollination with 1.25 g/L plus 1 g ascorbic acid compared with the traditional hand pollination.

These findings could be attributed to the reduction of fruit set as the pollen grains suspension concentration is reduced. This in turn leads to a reduction in the fruit retention, hence the fruit weight/bunch was reduced. The abovementioned results are in agreement with those obtained by Hussein et al. (1979), Shabana et al. (1998), Ragab et al. (2004) and El-Kady (2004).

Fruit Quality

Data in Table 3 clearly show that there was an improvement of the fruit physical characteristics in terms of increasing fruit weight and dimension and flesh percentage by using a dilution pollen grains suspension compared with the traditional hand pollination (control). The improvement of these characteristics was associated with the decrease of the used pollen grains suspension concentration from (20 g/L) plus 1 g ascorbic acid (T_3) to (0.62 g/L) plus 1 g ascorbic acid (T_8).

Such improvement was significantly increased with the use of pollen grains suspension at a concentration from 10 to 0.62 g/L compared to the control. Moreover, there are non-significant differences in these traits due to pollination by using either pollen grains.

Suspension (20 g/L) alone (T_2) or plus 1 g ascorbic acid (T_3) or traditional hand pollination (T_1), the best results dealing with fruit physical properties are observed on palms pollinated with a pollen grains suspension concentration at 1.25 g/L plus 1 g ascorbic acid (T_7). No significant differences in fruit physical properties were observed between the two pollen grain suspension concentrations namely 2.5 and 1.25 g/L (T_6 and T_7). The obtained fruit weights were 9.12, 9.17, 9.45, 10.09, 10.30, 10.88, 10.93 and 10.89 g as an average of the three studied seasons due to T1 to T8, respectively.

Such improvement of fruit physical properties, i.e., increasing the fruit weight and size might occur in response to using a diluted pollen grains suspension plus ascorbic acid for pollination. So, it could be stated that "there is a positive correlation between fruit weight and initial fruit set percentage".

These results could be due to the reduction on the fruit set percentage when using

the diluted pollen grains suspension. Such reduction in fruit set percentage causes a shortage in the number of fruits per bunch without changing the number of leaves that may induce a better supply of carbohydrates that are manufactured in the leaves. Such effects were similar to the fruit thinning effects in improving the physical fruit properties. So, it could be easy to identify the initial fruit set percentage which gave the considerable yield characterized by high fruit quality using either different hand pollination or fruit thinning methods.

Data in Tables 4 and 5 indicated that the pollination by diluted pollen grains suspension concentrations at 5 to 0.62 g/L leads to a significant improvement of the fruit chemical constituents in terms of increasing the total soluble solids and sugar contents and a reduction of the moisture content percentage compared to pollination by traditional hand pollination. The improvement of these fruit traits was associated with the reduction of pollen grain suspension concentrations from 20 to 0.62 g/L.

The reduction of the fruit moisture content is very necessary for improving the quality of such cultivars and resulted in an increase in packable yield.

These findings might be due to a reduction in the fruit set percentage by using the diluted pollen grain suspension. Such reduction in fruit setting was effective in lowering the competition that may occur between fruits and induce adequate carbohydrates and other essential foods for the residual ones consequently enhancing the fruit maturity and improving its contents of total soluble solids and sugar contents. So, it could be said that the use of a diluted pollen grain suspension has a similar effect like the fruit thinning on improving fruit quality.

These results were supported by the results of Ashour et al. (2004), Al-Sabahi et al. (2006) and Alabri et al. (2006) who recommended using 0.5 g pollen grains/L of H_2O concentration to pollinate 'Fard' and 'Jabree' date palms and 0.1 g pollen grains/L of H_2O for 'Helaly Oman' date palm. In addition, they found that such a pollen grain suspension concentration had an insignificant effect on fruit set percentage and total yield/palm, whereas it improved the physical characters of fruits, especially fruit flesh and fruit weight.

In regard of the previously mentioned results, it can be recommended that pollination of the 'Saidy' date palm using pollen grain suspension concentrations at 2.5 g/L plus 1 g ascorbic acid was sufficient to get a high yield with good fruit quality. The advantages of such a pollination method is the reduction of labor and duration of pollination, both contributing to the reduction of the cost of pollination. Furthermore, it does not require highly trained labor as with the traditional technique. It ensures the possibility of pollinating a palm several times in a short period of time. Moreover, allowing the use of a mixture of pollens originating from different sources, thus ensures good fertilization, and eliminates the risk of accidents occurring as with the old method of climbing a palm several meters high.

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Tables

Table 1. Temperature (°C) and relative humidity (%) at El-Kharga region during pollination periods of 'Saidy' date palms in the 2005, 2006 and 2007 seasons.

		2005			2006			2007		
		Temperature (°C)		R-humidity	Temperature (°C)		R-humidity	Temperature (°C)		R-humidity
		min	max	(%)	min	max	(%)	min	max	(%)
10	Mar.	16	24.2	25	6.8	22.6	52	14.8	34.1	32
11	Mar.	11.6	21.2	24	9.6	24.6	53	18.4	31.2	32
12	Mar.	9.6	21.6	35	6	29	56	18.5	29.2	36
13	Mar.	8.6	22.8	41	8.6	30.4	60	18.4	27.6	45
14	Mar.	9.8	23.8	35	9.9	31.4	62	13	25.8	44
15	Mar.	11.8	26	30	16.2	27.6	51	10.4	21.4	42
16	Mar.	9.2	24.6	37	10.6	27	55	7.6	22.6	44
17	Mar.	12	25.2	44	9.8	25.7	56	8.2	23.6	45
18	Mar.	13.6	25.8	43	9.8	26.8	57	10.8	23.6	50
19	Mar.	12.1	27.2	44	8.4	33.2	40	6.8	28.2	52
20	Mar.	8.6	28.6	43	13	32.4	48	9.6	33.6	35
21	Mar.	8.8	30	35	16.6	36	38	11.6	31.6	38
22	Mar.	11	31.6	38	14.8	37.4	31	12.6	33.2	30
23	Mar.	12.3	30.7	32	18.7	38.6	28	15.6	37.8	24
24	Mar.	14.6	26.6	40	15.2	39.4	31	17.2	25	20
25	Mar.	12.2	27.4	42	17.8	28.4	40	14.4	26	32
26	Mar.	12.2	28.2	38	11	29.2	46	12.4	25.2	39
27	Mar.	11.4	29.2	39	14.6	32	46	11.2	26	44
28	Mar.	13.6	32.2	32	15.8	26	57	9.8	32	45
29	Mar.	9	33.4	33	12.4	24.4	52	11.4	36	35
30	Mar.	10.6	36.6	35	8	27.6	54	16	36.2	23
31	Mar.	14.2	34.4	22	10.6	29.8	47	17.6	38.4	30
1	Apr.	13	36	23	10	27.4	46	19.1	29.4	28
2	Apr.	15.6	27	30	11.3	28.2	44	15.6	27.4	40
3	Apr.	13.6	25.4	37	13.5	29.9	49	15	27.4	41
4	Apr.	12	25.2	36	14.4	32	39	10.3	33.4	38
5	Apr.	13.8	30	33	15	29.4	30	16.2	39.4	28
6	Apr.	15.6	35.6	29	15.7	28.8	26	16.8	42.2	31
7	Apr.	17.6	44	29	17.1	33.2	31	19	43.4	26
8	Apr.	19.7	31.8	32	17.5	36	27	19.2	30.4	44

Source: El-Kharga Oasis Meteorological authority station.

Table 2. Effect of different pollen grain concentrations with or without ascorbic acid in fruit set and fruit weight/ bunch (kg) of 'Saidy' date palm during the 2005,2006 and 2007 seasons.

Treatment		Characteristics							
		Fruit set (%)				Fruit weight/ bunch (kg)			
		2005	2006	2007	Mean	2005	2006	2007	Mean
Hand pollination (traditional)	T_1	72.00	75.30	76.74	74.67	9.31	9.65	9.86	9.61
20 g/L pollen	T_2	72.68	74.90	76.63	74.74	9.42	9.65	9.90	9.66
20 g/L pollen +1.0 g ascorbic acid	T_3	72.87	75.30	76.72	74.96	9.74	10.03	10.3	10.02
10 g /L pollen +1.0 g ascorbic acid	T_4	62.25	65.30	65.91	64.47	8.93	9.20	9.40	9.18
5 g/L pollen +1.0 g ascorbic acid	T_5	55.21	58.40	59.62	57.73	8.69	8.98	9.10	8.89
2.5 g/L pollen +1.0 g ascorbic acid	T_6	52.36	55.60	55.81	54.59	8.06	8.34	8.40	8.77
1.25 g/L pollen +1.0 g ascorbic acid	T_7	42.43	44.9	45.89	44.41	7.01	7.24	7.30	7.18
0.62 g/L pollen +1.0 g ascorbic acid	T_8	24.24	25.90	26.93	25.70	4.25	4.43	4.55	4.41
LSD 5%		2.20	1.97	3.63	2.58	0.92	0.75	0.82	0.75

Table 3. Effect of different pollen grain concentrations with or without ascorbic acid on weight, pulp weight % and dimensions of 'Saidy' date fruits during the 2005, 2006 and 2007 seasons.

Treatment	Characteristics															
	Fruit weight (g)				Pulp weight (%)				Fruit length (cm)				Fruit diameter (cm)			
	2005	2006	2007	Mean	2005	2006	2007	Mean	2005	2006	2007	Mean	2005	2006	2007	Mean
T_1	9.25	9.12	8.98	9.12	85.82	86.09	85.90	85.94	3.46	3.36	3.36	3.39	2.04	2.09	2.06	2.06
T_2	9.38	9.17	8.95	9.17	85.82	86.17	86.09	86.04	3.48	3.37	3.35	3.40	2.04	2.09	2.06	2.06
T_3	9.63	9.41	9.30	9.45	86.28	86.61	86.32	86.40	3.62	3.46	3.47	3.52	2.07	2.12	2.08	2.09
T_4	10.30	9.95	10.02	10.09	86.94	87.17	86.93	87.01	3.87	3.72	3.66	3.75	2.11	2.16	2.15	2.14
T_5	10.60	10.24	10.11	10.30	87.01	87.38	87.00	87.10	4.03	3.84	3.75	3.87	2.12	2.17	2.16	2.15
T_6	11.20	10.80	10.61	10.88	87.18	87.71	87.13	87.33	4.10	3.90	3.85	3.95	2.15	2.21	2.21	2.19
T_7	11.20	10.95	10.61	10.93	87.18	87.71	87.21	87.37	4.15	4.01	3.92	4.03	2.16	2.22	2.22	2.20
T_8	11.20	10.87	10.59	10.89	87.16	87.68	87.21	87.35	4.15	4.01	3.92	4.03	2.16	2.22	2.22	2.20
LSD 5%	0.28	0.26	0.35	0.43	0.83	0.65	0.58	0.76	0.15	0.11	0.13	0.13	0.04	0.03	0.06	0.06

Table 4. Effect of different pollen grain concentrations with or without ascorbic acid on total soluble solids percentage and fruit moisture % of 'Saidy' date fruits during the 2005, 2006 and 2007 seasons.

Treatment	Characteristics							
	TSS (%)				Fruit moisture (%)			
	2005	2006	2007	Mean	2005	2006	2007	Mean
T_1	78.14	78.44	77.88	78.15	14.40	14.20	14.95	14.51
T_2	78.38	78.25	77.81	78.15	14.15	14.35	15.05	14.52
T_3	79.50	78.69	77.88	78.69	13.30	13.98	14.90	14.06
T_4	79.80	78.94	78.60	79.11	13.20	13.70	14.15	13.68
T_5	80.35	79.69	78.94	79.66	12.60	13.05	13.75	13.13
T_6	80.90	80.25	79.63	80.26	12.05	12.60	13.05	12.57
T_7	81.30	80.50	79.81	80.54	11.60	12.35	12.90	12.28
T_8	81.25	80.38	80.00	80.54	11.48	12.60	12.80	12.29
L.S.D. 5%	1.15	1.11	1.18	1.08	1.15	1.11	1.18	1.08

Table 5. Effect of different pollen grain concentrations with or without ascorbic acid on total sugars, reducing sugars and non reducing sugars of 'Saidy' date fruits during the 2005, 2006 and 2007 seasons.

Treatment	Characteristics											
	Total sugars				Reducing sugars				Non reducing sugars			
	2005	2006	2007	Mean	2005	2006	2007	Mean	2005	2006	2007	Mean
T_1	73.42	73.93	72.90	73.42	65.18	65.50	64.75	65.14	8.24	8.43	8.15	8.27
T_2	73.69	73.93	72.78	73.47	65.30	65.53	64.75	65.19	8.39	8.40	8.03	8.27
T_3	74.61	74.13	72.88	73.87	66.15	66.03	65.23	65.80	8.46	8.10	7.65	8.07
T_4	74.85	74.38	73.50	74.24	66.35	66.68	65.95	66.33	8.50	7.70	7.55	7.92
T_5	75.22	74.93	74.13	74.76	66.53	67.50	66.86	66.96	8.69	7.43	7.28	7.80
T_6	75.81	75.13	74.55	75.16	67.36	67.55	67.35	67.42	8.45	7.58	7.20	7.74
T_7	76.16	75.20	74.75	75.37	67.60	67.95	67.60	67.72	8.56	7.25	7.15	7.65
T_8	76.31	75.15	74.85	75.44	67.75	67.90	67.63	67.76	8.56	7.25	7.23	7.68
L.S.D. 5%	0.60	0.66	0.58	0.71	0.61	0.58	0.38	0.66	0.38	0.41	0.35	0.43

Pollination of Date Palm (*Phoenix dactylifera* L. 'Lulu') with Pollen Grains Water Suspension

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Abstract

Pollination and thinning are critical processes in the date palm production chain that affect fruit development, quality and yield and regulate tree yearly bearing. Developing a pollination technique that results in an acceptable level of fruit set with a minimum amount of pollen grains and without a further need for thinning is critically required for date palm production, especially under arid conditions. During the 2006 and 2007 seasons, the effect of pollen grains-water suspension application at different concentrations on fruit set, yield and quality were examined on 'Lulu' date palm growing under Al-Ain oasis conditions, UAE. The results showed that the pollen grains-water suspension (PGWS) application at 0.5, 1, 1.5 g/L gave fruit set percentages of 75.6, 86.8 and 87.8%, respectively which was lower than the control (90.0%) (traditionally pollinated). As the mean of both seasons, the GWS application especially at 0.5 and 1.0 g/L significantly decreased both bunch weight (5.52, 6.21, 7.19 and 8.54 kg for 0.5, 1.0, 1.5 g/L and control, respectively) and the total yield per tree (49.71, 55.92, 64.74 and 76.89 kg/tree, for 0.5, 1.0, 1.5 g/L and control, respectively) at the tamer stage. Fruit quality characteristics especially fruit and flesh weight, length and diameter, TSS and dry matter concentration were slightly but significantly increased by the PGWS application treatments during the 2006 season. However, seed weight, acidity and vitamin C concentration were not affected by any of the pollination treatments. It is concluded that this is a pioneer study investigating the possibility to pollinate date palm flowers with pollen grains-water suspension to control fruit set in heavy bearing cultivars such as 'Lulu' to save further thinning cost and pollens of excellent males, and improving fruit quality. However, more research work is required to justify the concentration of grains-water suspension and the response of each date palm cultivar to this pollination technique.

INTRODUCTION

Pollination and thinning are critical processes in the date palm production chain that affect availability of assimilates, fruit development, quality and yield and regulate tree yearly bearing (Nixon, 1955; Hussein et al., 1993; Awad, 2006). Being a dioecious species, commercial date palm production necessitates artificial pollination which ensures enough fertilization and overcomes disadvantages of dichogamy and also reduces the number of required male palms. Artificial pollination could be realized according to a traditional method or by using a mechanized device (Zaid and de Wet, 1999). The most common and primitive pollination technique is to cut the strands of male flowers from a freshly opened male spathe and place 5-10 of these strands, lengthwise and in an inverted position, between the strands of the female inflorescence and tie the pollinated female cluster 5 to 7 cm from the outer end with a strip torn from a palm leaflet or a string (see Zaid and de Wet, 1999). Fruit set ranges between 85-95% for 'Lulu' as well as most other cultivars by such pollination technique. With such a high fruit set percentage, thinning

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(flower and/or fruit thinning) is a necessary process for improving fruit quality and increasing the marketable yield. Also, this pollination technique requires the availability of a large number of male spathes that sometimes are not available especially for early flowering season cultivars (Dowson, 1982). The male/female ratio in modern plantations is about 1/50 (2%). Hand or chemical thinning are commonly practiced in different ways and is normally practiced at flowering time (flower thinning) or shortly after the completion of fruit setting (fruit thinning) (see Awad, 2006; Zaid and de Wet, 1999; Al-Khateeb et al., 1993; El-Hamady et al., 1993). However, hand or chemical thinning is neither practical nor economic especially under such harsh conditions as in the Gulf region (Awad, 2006). Due to the general concern with respect to the use of chemicals in agricultural production, more safe and economic thinners or methods are critically required. In mechanical pollination, dilution of pollen concentration with wheat flour might be a way to regulate fruit set (El-Kassas and Mahmoud, 1986a; El-Mardi et al., 1995). Intended delay of pollination is another way of regulating fruit set since the receptivity of pistillate flowers decreases by delaying pollination to one or two weeks from female spathe cracking. However, the results seem inconsistent among seasons and cultivars (El-Kassas and Mahmoud, 1986b; Moustafa, 1998a; Shabana et al., 1998). Moreover, Reuveni (1970) found in the 'Deglet Nour' cultivar that the day of optimum receptivity varies among the different inflorescences of the same tree. It has been reported that water spray treatment (as a simulation of rainfall) after a specific time following pollination decreased fruit set and induced an acceptable level of thinning, especially in arid regions where rainfall is scarce at the pollination period (Awad, 2006). Accordingly, the aim of this study was to evaluate a new pollination technique by the application of pollen grains-water suspension at different concentration on fruit set and fruit quality of 'Lulu', a heavy bearing date palm cultivar. Our goal was to develop a pollination technique that should result in an acceptable level of fruit set with a minimum amount of pollen grains and without a further need for thinning, especially under such harsh conditions.

MATERIALS AND METHODS

Plant Materials and Experimental Procedure

During the 2006 and 2007 seasons, three uniform 'Lulu' date palm trees of 17 years old, growing in a sandy soil at the experimental orchard of the College of Food and Agriculture, United Arab Emirates University at Al-Ain, United Arab Emirates, were selected. At the middle of the flowering period (March 14th and 19th in 2006 and 2007, respectively), on each tree, four normal bunches that were just cracked on the same day or the day before were randomly selected and labeled. Pollens were collected from the spathes of the male cultivar 'Sekkaa', air dried at room temperature and pollen grains were extracted form strands, then suspended in water at the rate of 0.5, 1.0 and 1.5 g/L directly before the pollination process. Each of the four bunches on each tree were treated with one of the following treatments: traditional pollination (control) by placing 7 strands of male flowers, lengthwise and in an inverted position, between the strands of the female inflorescence and tie the pollinated female cluster 5 cm from the outer end with a strip torn from a palm leaflet, pollination with pollen grains-water suspension (PGWS) spray at 0.5, 1.0 or 1.5 g/L. The PGWS was applied to the bunches with a plastic hand sprayer (Matabi Style 1,5 Sprayer-1L, Goizper, Spain) by targeting the cone nozzle close to the bunch. The pressure during the delivery of the suspension was about 30-40 PSI. Each bunch received about 100 ml of suspension. The same male cultivar was used during both seasons of the study. All the other developed bunches on each tree were subsequently pollinated in a similar way as for the control bunches. Shortly after pollination, the number of bunches was adjusted to 9 bunches per tree.

Fruit Set Percentage

In both the 2006 and 2007 seasons, at the middle of the kimri stage (about 7-8

weeks from pollination), 20 strands per bunch were randomly selected and labeled. The number of attached fruitlets and the number of dropped fruitlets were counted and the percentage of fruit set was calculated.

Bunch Weight and Total Yield per Tree

In both the 2006 and 2007 seasons, the total bunch weight at the tamer stage (about 22 weeks from pollination), were recorded for each bunch. The total yield (kg/tree) was calculated by multiplying the mean bunch weight by the total bunches per tree.

Fruit Qality Measurements

Only in the 2006 season, at the tamer stage, 20 fruits per tree (from all bunches) were collected and immediately transported to the Horticulture Laboratory at the College of Food and Agriculture for quality measurements. Fruit, flesh and seed weight (g), diameter and length (cm), and dry matter percentage were recorded. Total soluble solids (TSS) were measured in fruit juice with a hand refractometer. Titratable acidity was determined in juice by titrating with 0.1 N sodium hydroxide in the presence of phenolphthalein as indicator (Ranganna, 1979), and the results were expressed as a percentage of malic acid. Ascorbic acid (vitamin C) was measured, according to Ranganna (1979), by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye. The results were expressed as mg/100 ml juice.

Statistical Analysis of Data

All data were statistically analyzed, as a completely randomized design with three replicates per treatment, by analysis of variance (ANOVA) using the statistical package MSTATC Program (Michigan State University, East Lansing, MI). Comparisons between means were made by *F*-test and the least significant differences (LSD) at P=0.05.

RESULTS

Because of similarity between the results of the two seasons (no significant interactions between seasons), data were presented as the means of both seasons for fruit set, bunch weight and yield per tree. Data of Table 1 and Figure 1 show that the pollen grains-water suspension (PGWS) application at 0.5, 1, 1.5 g/L gave a fruit set percentage of 75.6, 86.8 and 87.8%, respectively which was lower than the control (90.0%) (traditionally pollinated). In this respect, the lowest fruit set percentage was obtained with the PGWS application at 0.5 g/L which was significantly lower than all other pollination treatments. There were no significant differences among the other PGWS applications and the control.

At the tamer stage, the PGWS application especially at 0.5 and 1.0 g/L significantly decreased both bunch weight (5.52, 6.21, 7.19 and 8.54 kg for 0.5, 1.0, 1.5 g/L and control, respectively) and total yield per tree (49.71, 55.92, 64.74 and 76.89 kg/tree, for 0.5, 1.0, 1.5 g/L and control, respectively). In this respect, there were significant differences among all the applied pollination treatments (Table 1 and Fig. 1).

DISCUSSION

Pollination and thinning are critical processes in the date palm production chain that affect fruit development, quality and yield and regulate tree yearly bearing. Developing a pollination technique that results in an acceptable level of fruit set with a minimum amount of pollen grains and without a further need for thinning is critically required for date palm production, especially under arid conditions (Awad, 2006). The results of this study showed that a pollen grains-water suspension (PGWS) application at 0.5 g/L significantly decreased fruit set percentage, in contrast to 1.0 and 1.5 g/L which gave a fruit set percentage similar to the control (traditionally pollinated) (Table 1 and Fig. 1). Generally, ‘Lulu’ is classified as a heavy bearing date palm cultivar and fruit set ranges between 85-95% by the traditional pollination technique (Awad, 2006). With such a high fruit set percentage, thinning (flower and/or fruit thinning) is a necessary process

for protecting bunches breaks, improving fruit quality and increasing the marketable yield. Thus, the application of PGWS especially at 0.5 g/L resulted in an acceptable level of fruit set (75.6%) with a minimum amount of pollen grains and without a further need for thinning (Table 1 and Fig. 1). The effectiveness of the PGWS application was also consistent between the two years. Moreover, in this technique, the pollen grains of an excellent male might be stored and used for pollination of the early flowering season cultivars (Dowson, 1982). The total bunch weight and yield per tree were significantly decreased by the PGWS application (Table 1 and Fig. 1). It is known that yield is a result of combined factors such as fruit number, size, weight and other related variables (Al-Khateeb et al., 2001). The parameters of this factor are often proportionally inversely correlated with each other. One of the main objectives of bunch thinning is to obtain more uniform bunch sizes and increase fruit size and weight depending on the level of fruit set. In this experiment, the obtained levels of thinning, especially with the PGWS application at 0.5 and 1.0 g/L, had a significant positive effect on most fruit quality characteristics at the tamer stage (Table 2). This might be due to more availability of assimilates by lower percentage of fruit set (Nixon, 1955; Hussein et al., 1993; Awad, 2006). In confirmation to our results, it has been reported that fruit thinning increased fruit size and weight and sugar level but decreased total yield per tree of 'Deglet Noor' (Nixon, 1955), 'Seewy' (Moustafa, 1998b), 'Samany' (Hussein et al., 1993) and 'Khalas' (Al-Khateeb et al., 1993) date palm cultivars. In the current study, seed weight, acidity and vitamin C concentration were not affected by any of the pollination treatments (Table 2). It was also reported that, for some date cultivars under certain climatic conditions, thinning had no pronounced effect on fruit quality (Al-Bekr and Al-Azzaoui, 1965; Awad, 2006). Generally, cultivars which produce shorter and more round fruit such as 'Lulu' and 'Barhee' showed no or little response to thinning, in contrast to those that produce a more elongated fruit type (Marashi and Mousavi, 2006).

In conclusion, this is a pioneer study investigating the possibility to pollinate date palm flowers with a pollen grains-water suspension to control fruit set in heavy bearing cultivars such as 'Lulu' to save further thinning cost and pollens of excellent males, preventing bunches breaks, improving fruit quality and producing consistent results among seasons. However, more research work is required to justify the concentration of the grains-water suspension and the response of each date palm cultivar to this pollination technique.

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Tables

Table 1. Fruit set percentage, bunch weight and total yield per tree at the tamer stage of 'Lulu' date palm as affected by pollen grains-water suspension.

Treatments	Fruit set (%)	Bunch weight (kg)	Yield/tree (kg/tree)
Control (traditional pollination)	91.0	8.54	76.9
Pollen grains-water suspension at:			
0.5 g/lL	76.6	5.52	49.7
1.0 g/L	86.8	6.21	55.9
1.5 g/L	87.8	7.19	64.7
F-test	**	***	***
$LSD_{0.05}$	4.63	0.69	6.27

Data are the mean of 2006 and 2007 seasons. ** and ***, significant at level P=0.01 and 0.001, respectively.

Table 2. Fruit quality of 'Lulu' date palm at the *Tamer* stage as affected by pollen grains-water suspension.

Treatments	Fruit quality								
	Flesh weight		Seed weight	Fruit length		Fruit diameter	Dry matter	TSS	Acidity Vit. C
	(g)	(g)	(g)	(cm)	(cm)	(%)	(%)	(%)	(mg/100 ml juice)
Control (traditional pollination°	6.22	5.34	0.88	2.23	1.72	75.6	81.4	4.86	2.32
Pollen grains-water susupension at:									
0.5 g/L	7.10	6.20	0.90	2.48	1.96	83.2	88.3	4.95	2.44
1.0 g/L	6.91	6.11	0.80	2.45	1.89	80.7	82.8	4.91	2.36
1.5 g/L	6.27	5.45	0.82	2.33	1.78	71.6	80.4	4.85	2.34
F-test	***	***	NS	-	-	-	**	NS	NS
$LSD_{0.05}$	0.15	0.10	-	0.16	0.18	1.88	1.39	-	-

Data are for the 2006 season. NS, not significant; *, ** and ***, significant at level P = 0.05, 0.01 and 0.001, respectively; (-), not calculated.

Data of Table 2 show that during the 2006 season, fruit and flesh weight, length, diameter, and dry matter and TSS concentration were significantly higher at the PGWS application at 0.5 and 1.0 g/L compared to the other treatments. However, seed weight, acidity and vitamin C concentration were not affected by any of the pollination treatments (Table 2).

Figure

Fig. 1. Effect of the pollen grains-suspension application on fruit set of 'Lulu' date palm. From left to right, the concentration of pollen grains-suspension was 0.5, 1.0, 1.5 g/L and control.

Mineral Elements Concentration in Healthy and Infected Date Bunch Fading Disorder

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Keywords: date bunch fading disorder, mineral nutrient

Abstract

Date bunch fading is a new disorder that caused heavy damage to the Iran's date palm plantations in recent years. It was first reported from Kahnodge area in Kerman province on the 'Mozafati' cultivar in 1989 and gradually distributed to the other main date producing provinces such as Hormozgan, Bushehr, Sistan and Baluchestan, Fars and Khuzestan. This disorder is often observed on soft and mid-ripening cultivars such as 'Mozafati', 'Kabkab', 'Mordaseng', 'Kalotae', 'Sayer', 'Barhee', 'Khasee', etc. Unfortunately most of these cultivars are trading and prevailing cultivars of Iran. Due to the high economic importance of this disorder many projects have been carried out in different fields in order to determine the causal agent. One of these projects was determination of nutrient elements in leaf and fruit of healthy and infected date palm to date bunch fading. In this study two orchards were selected and twenty 15-years-old trees of date palm ('Khasee') were used from each orchard. Fruit samples were collected at 6 stages, each stage consisting of 25-30 fruits at random basis from each date palm from 20th June to 15th Aug. Leaf samples were collected at 4 stages, each stage consisting of 25-30 leaflets consisting of sampling the middle pair of leaflets from the second row leaves on the mid position of rachis at random basis from each date palm from 30th March to 15th Aug. Then they were transported from the orchard to the laboratory. Healthy and disordered date palms were distinguished in the orchard after incidence of disorder. Due to the number of samples and different infection on each palm (the infection percent was 0 to 100) samples in a relation of 6 completely infected and 6 completely healthy date palms were analyzed for the concentration of nutrients such as N, P, K, Ca, Mg, Fe, Cu, Zn and Mn. The concentration of mineral elements in healthy and infected date palms were compared with the T-test with MSTATC and the fluctuation of mineral nutrient concentration was graphed. The results showed that:

- **From the observed concentrations it showed that healthy date palms had a significantly higher concentration of P, K and Zn of fruit and P of leaf than the infected date palms.**
- **Although there were no significant differences in N, Fe, Cu and Mn of fruit and N, K, Fe, Zn, Cu and Mn of leaf in healthy and infected date palms, the graphs showed that healthy date palm had higher N, Fe, ZN and Cu of fruit, Cu of leaf and lower Mn concentration of leaf and fruit than infected date palms.**

INTRODUCTION

Date bunch fading disorder (DBFD) is a new disorder and one of the most important problems which caused economic damage in Iran's date palm plantations in recent years. It was first reported from Iran and the world (Karampour et al., 2006). It was first reported from Kahnodge area in Kerman province on the 'Mozafati' cultivar in 1989 and gradually distributed to the other main date producing provinces such as Hormozgan, Bushehr, Sistan and Baluchestan, Fars and Khuzestan. This disorder is often observed on soft and mid-ripening cultivars such as 'Mozafati', 'Kabkab', 'Mordaseng', 'Kalotae', 'Sayer', 'Barhee', 'Khasee', etc. Unfortunately most of these cultivars are trading and prevailing cultivars of Iran. The worst DBFD damage happens during the fruits' transition

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from the khelal to rutab stage. This disorder occurs only in the generative maturing tissues, starting with light yellow lesions on peduncles (the main stalk of bunch), and gradually developing to longitudinal pale brown strips on the whole peduncle. Date fruits wilt, usually from the bottom of the strand up, and then the pedicels, peduncles and whole bunch will dry. At the very last the fruits will dry and severely defoliate (Karampour et al., 1999, 2002, 2006; Karimipour et al., 2002; Mirzaei et al., 2001). Reports of the amount of damage in different regions and in different years have varied from 0 to 80 percent loss of yield, but the mean has been 30-50% (Karampour et al., 1999, 2002; Karimipour et al., 2002; Mirzaei et al., 2001). The disorder has been related to several variables, including the environment factor (Karampour et al., 1999, 2002, 2006; Karimipour et al., 2002; Mirzaei et al., 2001; Poozesh et al., 2004; Rahkhodaei, 2005; Roosta, 2003; Sarhadi et al., 2004) cultural practice (Azadvar et al., 2005; Izadi et al., 2005; Sarhadi et al., 2004), a nutritional imbalance or deficiency (Mohebi, 2007; Roosta, 2003). In studies conducted, increased N fertilization increased the incidence of the disorder (Mirzaei et al., 2001), several fungi species were isolated from infected bunches in different cultivar and locations, but their pathogenic effects have not yet been confirmed (Alavi et al., 2004; Karampour et al., 2006). Interfering of other biotic agents such as bacteria, viruses, mycoplasma and MLO_s on incidence or development of disorder has not been confirmed yet (Azadvar et al., 2003; Karampour et al., 2006). Environmental factors, especially blowing hot and dry winds, sudden drop of relative humidity (RH <20%), high temperature (T_{max}>45°C) have had significant effects on incidence and progress of the disorder (Poozesh et al., 2004; Rahkhodaei, 2004). Intercropping with alfalfa and sorghum in infected date plantation has been reported effective in reducing the disorder damage (Izadi et al., 2005; Azadvar et al., 2005). Disorder decreased with increase of date palm age (Rahkhodaei, 2005). Calcium sulfate and chloride injection did not significantly effect but calcium nitrate significantly decreases the disorder (Ghaffari nejad et al., 2005). The disorder decreased in the infected gardens in which macro and micro nutrients were applied (Sabbah et al., 2005).

Comparing the concentrations of specific nutrients in healthy fruit with those in disordered would help to elucidate the role of mineral nutrition in DBFD. The purpose of this study was to compare the concentration of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn in the fruit and determine if there is a relationship between these fruit mineral element concentrations and the occurrence of DBFD in 'Khasee' date palm fruit throughout fruit development.

MATERIALS AND METHODS

This study was conducted during the 2004 fruiting seasons in a commercial orchard in Behbahan (30°6'N;50°14'E, elevation 313 m) the orchard was established on a sandy loam soil. It contains 19% clay, 54% sand, 27% silt and has a pH of 7.7 (USDA 1996). Daytime temperatures during the experimental period averaged 30.1°C and the total rainfall amount was 21.5 mm, with a peak of 11 mm in 4 April.

Plant Material

Twenty 15-year-old trees of date palm ('Khasee') were used in the study. 2 kg urea (46% N) per palm was applied during the current year.

Sampling

25-30 fruit at random basis were collected from each palm on 20 June, 5 July, 20 July, 25 July, 30 July, 5 August, 10 August and 15 August. The samples were placed in polythene bags in an ice box, then they were transported from the orchard to the laboratory.

Processing of Samples

Collected fruit were washed in a detergent solution, rinsed in water, washed in 0.6 M HCl and rinsed twice in distilled water as described by Schaffer et al. (1988). After

washing, the fruit were weighed and oven-vacuum dried for 72 hours. Dried samples were ground in a cyclone mill. For N determination 0.2 g of ground tissue was weighed and placed in a 100-ml digestion tube in which 2 g of Kjeldahl mixture and 5 ml H_2SO_4 were added, glass funnels were placed on the tubes and the tubes and funnels were placed on a preheated aluminum digestion block at 250°C for 1h. The temperature of the digestion block was raised to 380°C for an additional 3-hours period. After the tubes cooled to room temperature, 5 ml of distilled water was added to each tube and the preparation was agitated with a vortex mixer. The digested material was transferred to a 100-ml volumetric flask and the content was vigorously mixed and filtered through Whatman #1 filter paper into a 20-ml polyethylene scintillation vial (Hanlon et al., 1994). The nitrogen concentration was determined by the total Kjeldahl nitrogen method. For P, K, Ca, Mg, Fe, Zn, Cu and Mn determination, 1 g of ground tissue was weighed and ashed at 500°C. The ashes samples were digested with 5 ml of 6 M HCl and brought to 50 ml with deionizer water in a polyethylene volumetric flask. The preparation was shaken and filtered through Whatman #1 filter paper into a 20-ml polyethylene scintillation vial. P was determined by spectrophotometer against blank in 640 nm wavelength. K was determined by flame photometer against blank in 768 nm wavelength. Ca, Mg, Fe, Zn, Cu and Mn were determined by atomic absorption Varian 221.

Determination of DBFD

Healthy and disordered date palm were distinguished in the orchard after incidence of disorder (16 August). The damage rate of DBFD on each palm was 0 to 100%.

RESULT

Due to number of samples and different infections on each palm (the infection percent was 0 to 100) samples in relation with 6 completely infected and 6 completely health date palms were analyzed for the concentration of nutrients such as N, P, K, Ca, Mg, Fe, Cu, Zn and Mn. The concentration of mineral elements in healthy and infected date palms were compared and the fluctuation of the mineral nutrient concentration was graphed (Figs. 1-9).

Mineral Elements

There were no significant differences in fruit nitrogen concentrations between healthy and infected date palms in the different stages of sampling.

There were no significant differences in fruit P concentrations between healthy and infected date palms in the 1st and 2nd stage of sampling. Concentrations of P in healthy fruit were significantly higher than those in disordered fruit in the 3rd, 4th, 5th and 6th stage of sampling.

There were no significant differences in fruit K concentrations between healthy and infected of date palm in the 1st and 2nd stage of sampling. Concentrations of K in healthy fruit were significantly lower than those in disordered fruit in the 3rd stage of sampling. Concentrations of P in healthy fruit were significantly higher than those in disordered fruit in the 4th, 5th and 6th stage of sampling.

There were no significant differences in fruit Fe concentrations between healthy and infected date palms in the different stages of sampling except the 4th stage of sampling in which concentrations of Fe in healthy fruit were significantly higher than those in disordered fruit.

There were no significant differences in fruit Zn concentrations between healthy and infected date palms in the 1st, 2nd, 5th and 6th stage of sampling. Concentrations of Zn in healthy fruit were significantly higher than those in disordered fruit in the 3rd and 4th stage of sampling.

There were no significant differences in fruit Ca, Mg, Cu and Mn concentrations between healthy and infected of date palm in the different stages of sampling.

DISCUSSION

The incidence of date bunch fading disorder may be the result of a nutrient deficiency since there were few differences in the concentration of fruit mineral elements between healthy and disordered fruit. A number of studies have related fertilizer application to the incidence of the disorder in date palm. Ghaffari nejad et al. (2005) injected calcium sulfate and chloride in date palm trunks and observed that this injection did not have a significant effect on the occurrence of date bunch fading disorder meanwhile calcium nitrate significantly decreased date bunch fading disorder. It was also observed that the calcium concentration in leaf and bunch increased significantly. A study of the effect of optimum nutrient fertilization on the intensity of date bunch fading disorder failed to produce conclusive evidence for a relationship between optimum nutrient and date bunch fading disorder (sabbah et al., 2005).

Dialami and Pezhman (2005) carried out a study in order to evaluate the effect of foliar application of potassium sulfate, potassium nitrate and manganese sulfate on yield and quantity of date palm. It was observed that treatments had significant effects on some quantity characteristics such as fruit fresh weight, length, diameter and volume. Rousta (2004) carried out a study of foliar application of potassium sulfate and calcium chloride with or without applying micronutrients (to soil) on date bunch fading. In that study, the treatments were; control: only nitrogen fertilization application (T1); application of fertilizers containing the micronutrients (T2); foliar application of pure calcium chloride (5 kg/1000 L) (T3); T2+T3 (T4); foliar application of pure calcium chloride (5 kg/ 1000 L) (T5); and T2+T5 (T6). Foliar applications were carried out at 2, 4, 10 and 15 weeks after pollination. The results showed that foliar application of potassium sulfate and calcium chloride with or without micronutrients was more effective in decreasing the damage of the disorder. The treatments containing potassium decreased the percent of faded fruits and bunch and subsequently increased the yield significantly. Foliar application of potassium sulfate with or without adding micronutrients lowered the percent of faded bunches from 69% in control to 19.7 and 21.2% and faded fruits from 66.5% in control to 7.5 and 10.4% and increased the yield by 46 and 65%, respectively. There is almost no information on the relationship between mineral concentration in date palm fruit throughout fruit development and the incidence of the disorder. During the accelerated growth phase of date palm fruit cell enlargement takes place. During this period, excessive K in the fruit may accentuate the enlargement process and, consequently, contribute to weakening of the structural arrangement of the cell wall polysaccharides. Higher K concentration in the healthy fruit may also have resulted in synthesis of enzymes and decreased activity of these enzymes in the disordered fruit. This decreased activity of enzymes results in the breakdown of the starch molecules, cell wall softening and also cell wall deterioration. These results are in agreement with observations made by Rousta (2004) who observed healthy date palm had higher K concentration than disordered date palm. Healthy fruit and leaves contained a higher P concentration than disordered fruit. These results are in agreement with observations reported by Ghaffari nejad et al. (2005). Phosphorylases are among the enzymes involved in starch degradation by adding phosphate to glycocidic molecules forming monosaccharide phosphate (Smith, 1993). The high P concentration observed in the healthy fruit tissues are related to an increased demand for phosphate based substances such as ATP in the ripening tissues due to an increased respiratory rate and/or accelerated degradation of starch molecules, making the healthy fruit stronger sinks for P than disordered fruit.

CONCLUSIONS

A relationship between fruit K concentrations and date bunch fading disorder was found. Thus this study does support the hypothesis that date bunch fading disorder results from K deficiency. Further studies are needed to elucidate the role of mineral nutrition in the development of date bunch fading disorder. Such studies should include the use of radioactive markers for K and possibly other elements so that the incorporation of these

elements in different tissues of the fruit could be followed. Fruit nutritional studies, wherby specific elements could be withheld to try to induce the disorder, may be essential to determine the role of mineral nutrient elements in date bunch fading disorder. Except for K, P and Zn there were significant differences in mineral element concentrations. Between disordered and healthy fruit there were no significant differences in the other mineral concentrations. These results indicate that with nutritional imbalance date bunch fading disorder may appear. It is also possible that an unknown factor triggers the biochemical processes that result in those elevated K and P concentrations in the disordered fruit.

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Figures

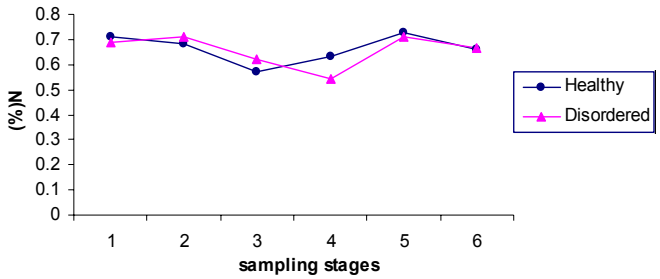

Fig. 1. Mean of N concentration in the healthy and disordered fruits of date palm.

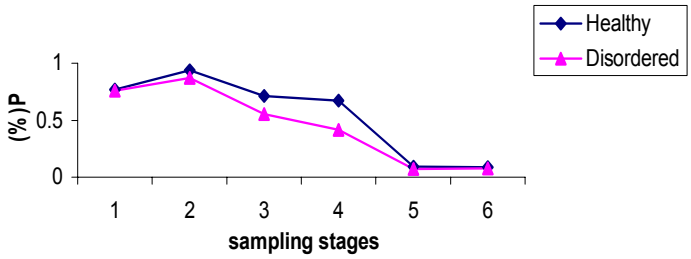

Fig. 2. Mean of P concentratiob in the healthy and disordered fruits of date palm.

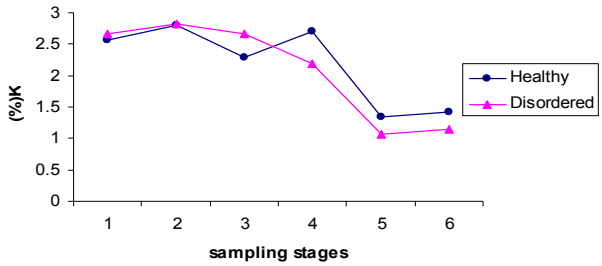

Fig. 3. Mean of K concentration in the healthy and disordered fruits of date palm.

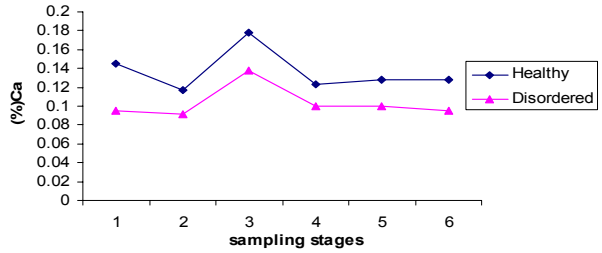

Fig. 4. Mean of Ca concentration in the healthy and disordered fruits of date palm.

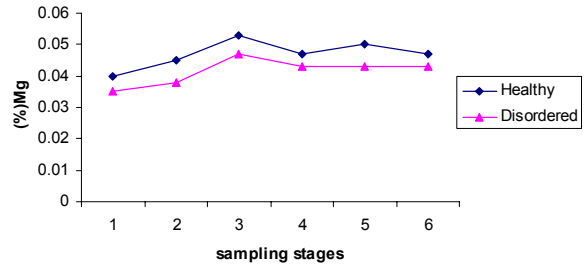

Fig. 5. Mean of Mg concentration in the healthy and disordered fruits of date palm.

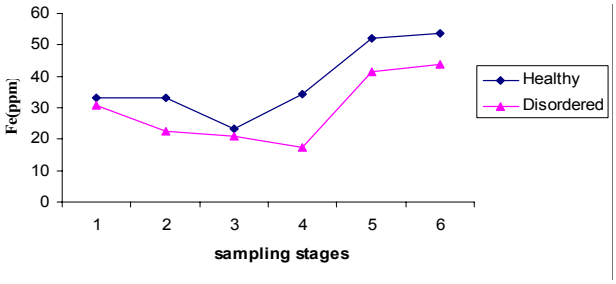

Fig. 6. Mean of Fe concentration in the healthy and disordered fruits of date palm.

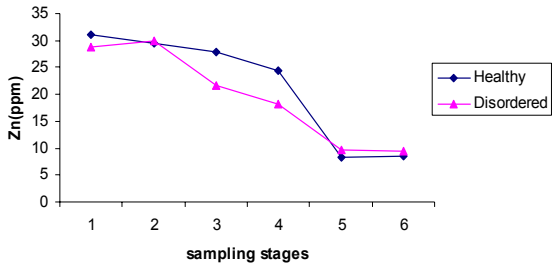

Fig. 7. Mean of Zn concentration in the healthy and disordered fruits of date palm.

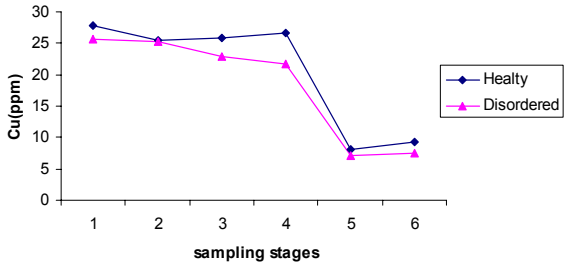

Fig. 8. Mean of Cu concentration in the healthy and disordered fruits of date palm.

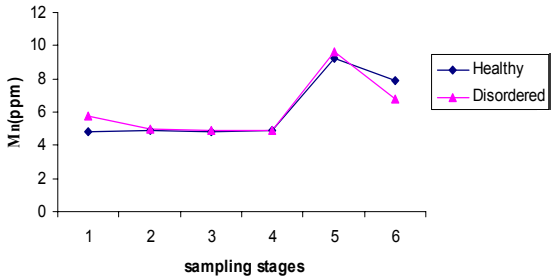

Fig. 9. Mean of Mn concentration in the healthy and disordered fruits of date palm.

Increasing Yield and Fruit Quality of 'Sayer' Date Palm with Application of Optimum Levels of Nitrogen, Phosphorus and Potassium

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Keywords: date palm, yield, fruit quality, nitrogen, potassium, phosphorus

Abstract

Date palm (*Phoenix dactylifera*) is one of the most important horticultural crops in Khuzestan Province. Among the date palm cultivars in this province 'Sayer' is one of the most commercial cultivars. The low annual average of yield and fruit quality is a problem for date growers. On the other hand, most of the date growers are not applying chemical fertilizers or applying them in improper amounts. Due to the importance of date palm and so the importance of mineral elements especially nitrogen, phosphorus and potassium in increasing the yield and fruit quality, determination of optimum levels of them for date palm fertilization is a necessity. In order to increasing yield and fruit quality of 'Sayer' date palm, this experiment with twelve treatments and four replications was carried out in a randomized complete block design on 48 'Sayer' date palm during four years in Khuzestan Province. Treatments consisted of three levels of nitrogen (N_1=700, N_2=1000 and N_3=1300 grams tree^{-1}), two levels of phosphorus (P_1=500 and P_2=650 grams tree^{-1}) and two levels of potassium (K_1=1000 and K_2=1300 grams tree^{-1}). The sources of nitrogen, phosphorus and potassium were urea, triple super phosphate (TSP) and potassium sulfate, respectively. These treatments along with 20 kg of manure and chemical micronutrient fertilizers based on general recommendation were applied for each tree in winter by localized placement method (Chalkood). The number of 20-25 leaflets from the middle of the leaf in the second row was picked up, and mineral nutrients of them were determined, each year. At harvesting time, plant parameters such as yield, concentration of mineral elements in leaf, average of weight, length, diameter and volume of fruit flesh, weight of stone and weight ratio of fruit pulp to its stone, pH, acidity, brix, reducing sugar, total sugar in fruits were determined. Data were analyzed with the MSTATC statistical program and means of data were compared with Duncan's Multiple range test. The results showed that application of 700 g N, 500 g P_2O_5 and 1300 g K_2O for each tree caused the highest yield and fruit quality. Therefore, annual application of these is recommended to date growers.

INTRODUCTION

Khuzestan Province is considered as one of the important regions of date production in Iran. According to the agriculture statistic book of Iran, the mature date plantation area and production in Khuzestan province are 26,000 ha and 142,000 tons (2003-2004). Among the date palm cultivars in province, 'Sayer' with more than 68 percent of date palm plantation area is one of the most commercial cultivars. The annual average of yield production for it is reported approximately 60-70 kg for each tree, while there is a higher production potential for this cultivar in this region. Now, palm-groves of this Province are managed traditionally. In this type of management, proper plant nutrition and soil fertility protection are not completely considered. Therefore, determination of optimum levels of fertilizers for this cultivar causes more yield and higher quality of date fruit, so could bring considerable income to farmers and is necessary. This experiment was done in order to study the effects of nitrogen, phosphorus and potassium on yield and fruit quality of 'Sayer' date palm and determination of optimum levels of chemical macronutrient fertilizers for it in Khuzestan Province. Karami

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(2007) observed that application of 800 g nitrogen and 375 g phosphorus for each tree caused the highest yield production in date palm 'Mordaseng' in Minab. Shahrokhnia (1992) reported that application of 800 g N and 650 g P_2O_5 per tree was the best fertilizer recommendation for date palm in Jahrom. Sinclair et al. (1981) investigated the effect of different fertilization levels on growth and chemical composition of date palm. They reported that application of 1100 g N and 800 g P_2O_5 for each fruitful tree caused the highest yield and fruit quality. Harhash (2000) recommended 1.5 kg potassium sulfate/palm/year in date palm. Bamiftah (2000) recommended 2 or 3 kg of potassium sulfate /palm/year for high yield and fruit quality in date palm 'Zaghloul'. Saleh (2008) showed that using 2.5 kg NPK (named as complete macro fertilizer) along with micronutrient fertilizers caused the highest yield and fruit quality. Dialami (2009) reported that application of proper amounts of nitrogen, phosphorus and potassium caused the best results on yield and fruit quality of date palm in Khuzestan province. Regarding the desirable effects of macronutrients application on yield and fruit quality and this fact that in most palm-groves in this province, fertilizers are not applied in proper amounts, it is necessary to determine the optimum levels and ratios of chemical fertilizers in order to increase the yield and improve fruit quality. Therefore, the main aim of this study is to determine the optimum levels and ratios of nitrogen, phosphorus and potassium in order to increase the yield and improve the fruit quality of 'Sayer' date palm.

MATERIALS AND METHODS

This experiment was conducted during four years on 48 fruitful, 15-year-old date palms in Khuzestan province. Before the application of treatments soil and water were sampled and analyzed (Tables 1 and 2). Treatments consisted of three levels of nitrogen (N_1=700, N_2=1000 and N_3=1300 grams tree^{-1}), two levels of phosphorus (P_1=500 and P_2=650 grams tree^{-1}) and two levels of potassium (K_1=1000 and K_2=1300 grams tree^{-1}). Treatments were: $T_1=N_1P_1K_1$, $T_2=N_1P_1K_2$, $T_3=N_1P_2K_1$, $T_4=N_1P_2K_2$, $T_5=N_2P_1K_1$, $T_6=N_2P_1K_2$, $T_7=N2P_2K_1$, $T_8=N_2P_2K_2$, $T_9=N_3P_1K_1$, $T_{10}=N_3P_1K_2$, $T_{11}=N_3P_2K_1$, $T_{12}=N_3P_2K_2$. The sources of nitrogen, phosphorus and potassium were urea, triple super phosphate and potassium sulfate, respectively. Half of the nitrogen and all of phosphorus and potassium was used in February. The rest of the nitrogen was applied in May, each year. These treatments along with 20 kg of manure and chemical micronutrient fertilizers based on general recommendation, consisting of application 150 g of zinc sulfate, iron sulfate, copper sulfate and manganese sulfate were applied for each tree in winter by localized placement method (Chalkood). Agro-technical practices such as pollination, thinning, irrigation and so on, were done according to the custom of the region. Each year, in May, the number of 20-25 leaflets from middle of leaf in second row was picked up, and mineral nutrients of them were analyzed. At harvesting time, plant parameter such as yield, average of weight, length, diameter and volume of fresh fruit, weight of stone and weight ratio of fruit pulp to its stone, pH, acidity, brix, reducing sugar, total sugar in fruits were determined. Data were analyzed with the MSTATC statistical program and means of data were compared with Duncan's Multiple range test.

RESULTS AND DISCUSSION

The presented data in Tables 3, 4, and 5 showed that some parameters of fruit quality such as concentration of nitrogen and phosphorus in leaf, diameter, pH, brix and acidity of fruit and stone weight were not affected by treatments. The effects of different fertilization treatments on the average yield/palm and fruit quality such as fresh weight, length and volume of fruit, pulp weight and weight ratio of fruit pulp to its stone as physical characteristics, total and reducing sugar in fruit and concentration of potassium in date palm leaf as a chemical characteristics were significant.

Yield

The present results indicated that fertilization treatments caused a significant increase in average of production yield in date palm 'Sayer'. Treatment 6, consisting of

using 700 g N, 500 g P_2O_5 and 1300 g K_2O for each tree, caused the highest average production yield (111.49 kg $tree^{-1}$) (Table 3). Desirable effects of some macro elements upon date palm yields were reported by other researchers. EI-Hammady et al. (1991) found that the highest yield and fruit quality of 'Seewy' dates were obtained by adding 2 kg potassium sulfate/palm yearly. Kassem et al. (1997) reported an increase in N and K contents of pinnate in date palm 'Zaghloul' due to an increase of potassium fertilizer rate, while Ca and Mg contents tended to decrease. Bamiftah (2000) recommended 2 or 3 kg of potassium sulfate/palm/year for high yield and fruit quality. The present results may be attributed to the physiological role of potassium in enhancing many metabolic processes such as carbohydrate formation, translocation and accumulation (Evans and Sorger, 1966; Marchner, 1986). Archer (1985) reported that translocation of photosynthetics depended on cell potassium concentration. The obtained results are in close agreement with those found by Abdalla et al. (1987), EI-Hammady et al. (1991), Shawky et al. (1999), Harhash (2000), Abdel-Nasser et al. (2000) and El-Shazly and Abdel-Nasser (2001). In addition to nitrogen, phosphorus is necessary for protein synthesis and energy carriers like ATP (adenosine tri phosphate) (Mengel and Kirkby, 1978), so, using these essential elements could increase the yield. Yield increase with using nitrogen and phosphorus fertilizers in date palm was also reported by Karami (2007), Sabbah (1993) and Bliss and Mathez (1983).

Fruit Quality as Physical Characteristics

Some physical characteristics of fruit such as fruit diameter and stone weight were not affected by treatments. Therefore, the results related to mentioned parameters were omitted. However, the present results indicated significant increments in fruit quality such as fresh weight, length and volume of fruit, pulp weight and weight ratio of fruit pulp to its stone as a result of fertilization with different levels of nitrogen, phosphorus and potassium. The results showed that fertilization treatments caused significant increasing in average fresh weight in date palm 'Sayer'. The highest average fresh weight of fruit (7.31 g) was achieved by treatment 6, in comparison with other treatments (Table 3). There were significant differences among treatments in pulp weight of fruit and higher pulp weight was seen in treatment 6. Increment in pulp weight of fruit could be due to improving cell size or cell number by nutrient elements. These findings are in harmony with Sourour et al. (1998). They found pulp weight of date fruit in the 'Samany' cultivar had significantly increased by using organic plus inorganic fertilizers in comparison with organic ones alone. Dialami and Pezhman (2005) reported foliar application of pure potassium sulfate (5 kg/1000 L) caused increased yield and fruit quantity characteristics such as weight, length, diameter and volume of fresh fruit, weight, length, diameter and volume of stone and pulp to stone ratio of date fruit of the 'Toory' cultivar. Fisher et al. (1959) mention that potassium is essential for fruit enlargement. In this experiment the application of different levels of nitrogen, phosphorus and potassium caused a significant increase in fruit length. The highest amount of fruit length (4.26 cm) was observed by applying treatment 6 in comparison with other treatments (Table 3). The weight ratio of fruit pulp to its stone was affected by using different fertilization treatments. The present results showed that treatments 2 and 6 caused the highest weight ratio of fruit pulp to its stone; 9.68 and 9.74, respectively. Similar results were obtained by El-Deeb et al. (2000) and Ismail (1999) on 'Hayany' date palm. They reported that artificial nitrogen fertilizer significantly increases the weight ratio of fruit pulp to its stone in fruit date. The fruit volume was affected by fertilization treatments. The highest amount of fruit volume (8.19 cm^3) was observed by applying treatment 6 in comparison with other treatments (Table 3). Overall, improvement that occurred in the physical characteristics could be attributed to effects of nutrients on the carbohydrate influx or plant growth regulators synthesis in growing fruits. Potassium plays an important role in pH stabilization, osmoregulation, enzyme activation, protein synthesis, stomata movement, photosynthesis, and cell extension (Läuchli and Pfluger, 1978). Moreover, potassium is an important solute in expanding cells (Marchner, 1986). These results are in agreement with those

obtained by EI-Hammady et al. (1991). Kein and Zaid (2005) mention that phosphorus plays a role in processes such as photosynthesis, respiration, vegetative growth, reproduction and maintenance of the genetic identity. It is also associated with cell division, root development and flowering.

Fruit Quality as Chemical Characteristics

Some chemical characteristics of fruit such as pH, brix and acidity of fruit were not affected by treatments. Therefore, the results related to the mentioned parameters were omitted. However, results indicated significant increments in fruit quality such as reducing sugar and total sugar in fruits as a result of fertilization with different levels of nitrogen, phosphorus and potassium. Application of different levels of nitrogen, phosphorus and potassium enhanced the amount of reducing sugar in date fruit in date palm 'Sayer' (Table 4). Increases in reducing sugar content could be due to the necessity of mineral elements for synthesis of sugar products and photosynthesis (Broschat, 1999; Mengalel and Kirkby, 1978). Other researcher (Bliss and Mathez, 1983; Sinclair et al., 1981) showed the desirable effect of different levels of nitrogen, phosphorus and potassium on increasing reducing sugar. The obtained results in this experiment are in agreement with those found by Saleh (2009) on 'Piarom' date palm. The total sugar percentage of 'Sayer' date fruit increased significantly by the application of fertilization treatments. These results are due to the fact that potassium activates the enzymes involved in sugar biosynthesis and helps in translocation of sugars (Evans and Sorger, 1966; Archer, 1985). In addition, Suelter (1970) mentioned that there are more than 50 enzymes which are stimulated by potassium. The obtained results appear to be in close agreement with the findings reported by Rizk (1987) on date palm 'Samany' and date palm 'Hayany'; El-Hammady et al. (1987) on date palm 'Sweey'; El- Deeb et al. (2000) on date palm 'Hayany'.

Mineral Element Concentration in Date Palm Leaf

The results showed there were no significant differences between fertilization treatments in effect on nitrogen concentration in date palm leaf but from the numerical viewpoint, treatment 6 and 1 showed the highest and the lowest nitrogen concentration, respectively (Table 5). Also there were no significant differences between the treatments in effect on phosphorus concentration but from the numerical viewpoint, treatment 10 caused the highest phosphorus concentration in date palm leaf (780 mg kg^{-1} dry wt) and treatments 1 and 5 showed the lowest phosphorus concentration (490 mg kg^{-1} dry wt), respectively (Table 5). According to Table 5, application of chemical fertilizers created significant changes in the potassium concentration in date palm leaf. The present results showed that treatments 2 and 6 caused the highest potassium concentration in the leaf of date palm (5808 and 6746 mg kg^{-1} dry wt) and treatment 1 showed the lowest potassium concentration (4550 mg kg^{-1} dry wt) respectively. Increasing leaf elemental contents due to fertilization may be attributed to the fact that chemical fertilization can improve the plants' ability to uptake mineral nutrients. Improving plant uptake reflects on increased vegetative growth and consequently improves efficiency for absorption and utilization of nutrients (Mangle and Kirkby, 1978; Abdel-Nasser and El-Shazly, 2001). These results are in harmony with those obtained by Perica et al. (1994) and Loupassaki et al. (1997). In general, in this experiment, it can be found that balanced application of nutrient elements such as nitrogen, phosphorus and potassium is required for optimum nutrition of date palm. Findings of this research showed that application of optimum nutrition of these essential elements had considerable effects on more yield production and improving the fruit quality in date palm 'Sayer'. So, determination of optimum levels of nitrogen, phosphorus and potassium fertilizers and application for them for this cultivar is a necessity and could bring considerable income to farmers. Therefore, in order to increase yield and improve fruit quality, annual application of 700 g N, 500 g P_2O_5 and 1300 g K_2O for each tree is recommended to date growers in Khuzestan province.

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Tables

Table 1. Soil sample analysis of the experimental site.

Soil depth (cm)	EC (ds m^{-1})	pH	OC (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})
0-30	2.16	7.7	0.83	12	173
30-60	2.74	7.6	0.65	8	155
60-90	2.09	7.7	0.60	8	147

Table 2. Water sample analysis of the experimental site.

EC (µmhos cm^{-1})	pH	SAR	Na	Ca	Mg	CO_3	HCO_3
			(meq L^{-1})				
2150	7.75	8.32	22.03	6.8	7.2	0	6.25

Table 3. Means comparison of effect of different treatments on yield, fresh weight, length, diameter, pulp weight and volume of fruit, weight of stone and weight ratio of fruit pulp to its stone.

Treatment	Yield (kg tree^{-1})	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Fruit volume (cm^3)	Fruit pulp weight (g)	Stone weight (g)	Weight ratio of fruit pulp to its stone
T_1	85.58b	6.86b	2.06a	3.62b	7.19b	6.68b	0.70a	9.29ab
T_2	97.58b	7.02ab	2.05a	3.85b	7.49ab	7.20ab	0.70a	9.68a
T_3	87.83b	7.01ab	2.05a	3.76b	6.83b	7.01ab	0.70a	9.28ab
T_4	92.09b	7.01ab	2.03a	3.71b	7.11b	7.01ab	0.71a	9.05b
T_5	86.17b	6.05ab	2.07a	3.76b	7.23b	6.05ab	0.69a	9.46ab
T_6	111.40a	7.31a	2.11a	4.26a	8.19a	6.31a	0.71a	9.74a
T_7	88.33b	6.05ab	2.07a	3.78b	7.48b	6.05ab	0.72a	9.35ab
T_8	92.58b	6.9ab	2.03a	3.78b	7.02b	6.99ab	0.70a	9.24ab
T_9	90.42b	7.12ab	2.04a	3.76b	6.94b	7.12ab	0.66a	9.52ab
T_{10}	89.17b	6.99ab	2.05a	3.76b	7.30ab	6.99ab	0.70a	9.22ab
T_{11}	88.50b	7.06ab	2.05a	3.72b	7.29ab	7.06ab	0.71a	9.38ab
T_{12}	88.70b	7.11ab	1.99a	3.68b	7.19b	7.11ab	0.70a	9.37ab

Table 4. Means comparison of effect of different treatments on pH, acidity, brix, reducing sugar and total sugar in fruit.

Treatment	Fruit pH	Fruit acidity (%)	Fruit brix (%)	Reducing sugar of fruit (%)	Total sugar of fruit (%)
T_1	6.00a	0.25a	77.88a	75.08a	66.86b
T_2	6.04a	0.27a	78.79a	64.64b	72.78a
T_3	6.00a	0.25a	76.54a	65.07b	65.73b
T_4	5.59a	0.26a	77.45a	64.64b	67.03b
T_5	6.01a	0.24a	76.78a	64.64b	65.84b
T_6	6.11a	0.27a	78.05a	69.71ab	69.32b
T_7	6.02a	0.26a	76.88a	65.07b	65.95b
T_8	6.02a	0.25a	78.04a	61.00b	67.61b
T_9	6.00a	0.25a	76.66a	65.85b	67.17b
T_{10}	6.05a	0.25a	77.38a	67.08b	66.02b
T_{11}	6.02a	0.26a	77.46a	67.08b	64.64b
T_{12}	6.11a	0.24a	77.96a	69.70ab	67.63b

Table 5. Means comparison of effect of different treatments on concentration of nitrogen, phosphorus and potassium in the leaf.

Treatment	Nitrogen (mg kg^{-1} dry wt.)	Phosphorus (mg kg^{-1} dry wt.)	Potassium (mg kg^{-1} dry wt.)
T_1	8270a	490a	4550b
T_2	8823a	522a	5808a
T_3	8274a	509a	4695b
T_4	8272a	499a	5126b
T_5	8420a	490a	4918b
T_6	9291a	521a	6746a
T_7	8672a	498a	4972b
T_8	8239a	492a	4800b
T_9	8700a	500a	5000b
T_{10}	8547a	780a	5000b
T_{11}	8376a	490a	4729b
T_{12}	8329a	492a	4671b

Case Study on the Trunk's Deformity of Date Palm Trees Used in the Street Landscape in Riyadh, Saudi Arabia

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Keywords: date palms trees, *Phoenix dactylifera*, deformation, street landscape

Abstract

The presence of deformations has been observed in different areas on the trunks of date palm trees, *Phoenix dactylifera* L., planted and used in the landscape of sites and streets in Riyadh, Saudi Arabia. This study was conducted on the trunks of date palms, to investigate their cases and identify factors that led to distortion. The study was done on street islands planted with date palm trees in five different districts of Riyadh city. Each island of the street was divided into three parts and twenty palm trees from each part were studied. This study showed that date palm tree trunks used in the landscape of the streets were affected by damage and deformity at different heights on the trunk. The percentage of deformed date palm trees' trunks in four studied districts reached (100%), while it was (70.3%) in the fifth "Al-Khozama" district. In all studied districts, most of the trunk's deformity of date palm trees started from the soil surface, and the length of the deformation area on the trunks varied among these districts. The highest value of the average length of the deformation area was (119.6 cm) while the lowest value was (28.7cm). The results also showed that the highest value of the percentage of deformation (length of deformed area/length of trunk) on the trunk was (11.46%), while the lowest value was (6.26%). In this study, various of fungous species (*Macrophomina phaseolina*, *Fusarium oxysporum, Fusarium solani*) were isolated, purified and defined. These fungi found on the leaf bases in most deformed areas were a result of high humidity leading to rot and decay. This study indicated that the distorted areas on date palm trunks could be due to the growth of lateral roots on the trunks, causing the separation of the leaf bases. The occurrence of this case is because of some irrigation systems used improperly and resulted in water reaching directly to the palm trunks.

INTRODUCTION

Date palm *Phoenix dactylifera* L. is the most important tree planted in the Kingdom of Saudi Arabia, which represents the historic symbol of the country cultivation and economy. It is one of the important wealth which is characterized by the Kingdom and constitutes the major part of its official emblem. The date palm trees since antiquity were the main source of food and development of many nations, especially in the Arabian Peninsula. The number of date palm trees planted in the Kingdom is close to 23.5 million trees, and the number of date palm trees planted in the Riyadh region is about 5.5 million trees (Ministry of Agriculture, 2009).

The palm trees are used in the landscape and beautification of the urban cities, where their presence complete the architectural formal view, take care of the aesthetic values, which are derived from the environment in the streets, plazas and gardens. So, they are highlighted as one of the traditional Arabic elements. Palm trees are important elements in the design and landscape of sites, for their distinguished properties and can be used as accent plants where each has its own character (Sayan, 2001). Date palm tree is the basic and the most important element in planting and landscape of streets at various locations in the Riyadh region, Saudi Arabia. It is suitable to the environmental conditions

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of the Kingdom in terms of high temperatures and scarcity of water and increased salinity, in addition to the aesthetic and landscaping aspects which they confer on sites that are planted (Al-Mana, 2007). Palm tree is characterized by its suitability for the landscape in the streets and the various sites. This is due to the nature of the palm's erect growth, its regular trunk and because it has no side branches obstructing the traffic or interfering with the buildings and other constructions at the sites. The most important special character of the date palm tree to be used in street landscape is its cylinder stem which is covered with the leaf bases in spiral form that give it a unique texture and picturesque view. The diameter of the date palm trunk reaches 40-90 cm depending on the variety, however, the trunk remains in the same thickness along the stem as long as the service operations are regular (Ibrahim and Kholeaf, 1998). While the palm tree increases in growth, its trunk increases in growth. At the completion of the leaf growth and becoming dry, the trunk reaches the highest degree of inflation (Albaker, 2002). The roots of date palm are able to grow on moist soil conditions for a certain period, but with the continuation of moisture in the soil for a long time, it becomes harmful to the roots and to the production of the dates (Zaid and Arias-Jimenez, 2002). The growth response of date palm trees varies according to the irrigation methods and different water levels (Amiri, 2007). There are many studies on irrigation methods for date palm trees (Aldakheel and Sheikhany, 2004; Bacha et al., 1997; Al-Amoud and Sharf, 1997). However, they did not address the negative effect of irrigation on the trunk especially in the case of using irrigation methods in improper practices. Deformed trunks of date palm trees have been observed in several locations and streets in the Riyadh region. The deformation on the trunks causes the palm trees to lose their sense of visual beauty when used in the landscape and also affects consensus and the harmony between the palm trees and the other plant units used in the landscape of the site. Therefore this study aims to identify the case of trunk's deformity of date palm trees planted and used in the landscape of streets in various districts of Riyadh region, and reasons leading to the deformation of these trunks.

MATERIALS AND METHODS

This study was conducted on the trunks of date palm trees, *Phoenix dactylifera* L. planted and used in the landscape of streets in the Riyadh region during 2009. Five streets "median islands" planted with date palm trees were randomly selected in various districts of Riyadh city as follows:

1. King Fahad district "Al- Imam Muhammad Bin Saud street".
2. Al-Gamaa district "Sheikh Hassan Bin Abdullah Al Al-Sheikh street".
3. Al-Khozama district "Prince Mishaal Bin Abdulaziz street".
4. Om Al-Hamam district "Prince Turki Bin Abdulaziz street".
5. Al-Nasseriah district "Prince Sattam Bin Abdulaziz street".

Each median island of the selected streets in the various districts was divided into 3 parts. The study was carried out on 20 palm trees from each part. The percentage of the deformed trunks of date palm trees in each district was calculated. The lengths of the deformed area "separation of leaf bases" on the trunks of date palm trees, and distance of the deformed area from the soil surface were measured. Heights of the trunks of studied date palm trees in the selected streets were also measured. The percentage of the deformity for each date palm trunk was calculated (length of the deformed area/trunk length ×100). All observations on the used irrigation methods were recorded for each street in the various districts. The pH of the irrigation water was 7, while the water salinity "EC" ranged from 2.5-4.5 (dS m^{-1}). The layout of the study was Complete Randomized Design (CRD). Data were analyzed by the analytical program SAS, using the revised LSD test at 5% level of probability to compare the mean values (Steel and Torrie, 1980). To find out which fungi species grow on the infected parts with rot at the bases of palm leaves, samples were collected from the infected leaf bases in all studied districts. The samples were isolated and analyzed in accordance with the biology method used for fungi extraction and purification (Rashed and Abd El-Hafeez, 2001). This work was done in the fungal and bacterial diseases laboratory, Department of Plant Protection,

College of Food and Agricultural Sciences, King Saud University.

RESULTS AND DISCUSSION

The data in this study show the case of the deformity on the trunks of date palm trees planted in the median islands of some streets in various districts of the Riyadh region (Table 1). The percentages of deformed trunks of date palm trees were 100% in all studied districts except the "Al-Khozama" district where it was 70.3%. The results of statistical analysis showed that the length of the deformed area on the trunks of date palm trees varies among the different districts in Riyadh region. It was found that the greatest average value of length of the deformed area was 119.6 cm in the "Om Al-Hamam" district "Prince Turki Bin Abdulaziz street", where it was observed that the date palm trees were irrigated by the bubbler method, in the addition of mobile irrigation tanks when water ceased. The smallest value of length of the deformed area was 28.7 cm in the "Al-Khozama" district, where it was observed that the irrigation of the date palm trees was by drip method, in addition to the mobile irrigation tanks. The results showed that most of the deformed areas on the trunks of date palm trees were starting at the soil surface in all districts. It is observed that these cases refer to the separation of leaf bases from the trunk and the growth of the lateral roots on it. The increased moisture leads to stimulation of lateral roots to come out in large groups on the trunk of the date palm. Usually the roots emerge from the tissue of the trunk at a height of 30 cm from the soil surface where they grow and extend under the leaf bases, so this stress leads to cracking and separation of leaf bases from the trunk (Maki et al., 1998). The results showed that the greatest average height of the deformed area from the soil surface in the "Al-Gamaa" district was 21.62 cm while the smallest average height was 0.4 in the "Al-Khozama" and "Al-Nasseriah" districts. However, most of the deformed areas were near or at the soil surface in the various districts. The results also showed that the percentage of deformation of date palm trunks (length of deformed area/length of trunk) was the highest in the "Om Al-Hamam" district (14.17%) and the lowest in the "Al-Khozama" district (6.26%). The percentage of deformation was greater in "Om Al-Hamam" than in the other districts (Fig. 1). The results of this study showed that the percentage of deformation on the trunks of date palm trees planted in the median islands in Riyadh region, varied according to the used irrigation method. The percentage of deformation obtained when using the bubbler irrigation method was greater than those obtained when either drip irrigation or flood irrigation was used (Fig. 2). For all the used irrigation methods, additional irrigation by mobile water tanks was applied when water ceased or by hoses connected to the fixed water outlets in median street islands. The percentage of deformation on the trunks of date palm trees was the lowest when using drip irrigation and the highest with the use of the bubbler irrigation or flood irrigation method. The reason for increasing the percentage of deformation when using the bubbler irrigation may be attributed to damage of its water outlets and so water reaching directly and continuously to the trunks at different heights. It also may attribute the cause of increasing the percentage of deformation when using bubbler or flood irrigation as a result of the cases which were observed when irrigation was applied directly by hand at the water cease. It was found through some studies that the use of the bubbler irrigation method gave an increase in vegetative growth of the date palm trees more than the other methods of irrigation (Amiri et al., 2007; Bacha et al., 1997). Bacha et al. (1997) explained that drip irrigation had led to increase the yield of date palm trees. Al-Amoud and Sharf (1997) showed that drip irrigation was the most suitable method for date palm trees. It works to save water, limits the growth of weeds, improves the quality of roots and reduces the attack of diseases. On the other hand, the bubbler irrigation method leads to accumulated water around the trunks of the date palm trees which are exposed to runoff, where it requires placing circular soil barriers around the palm trunk to prevent loss of water. It is observed that most cases of trunk's deformity of date palm trees appeared at the soil surface (Fig. 3). It is also observed that the deformations on the trunks appeared at different heights of soil surface (Fig. 4). This study showed that the apparent case of deformation on the trunks of date palm trees

resulted from excess moisture at the area of leaf bases, which leads to increased growth of lateral roots under the leaf bases, pushing and causing the separation of the leaf bases and then inflation of the trunk in this area. When there is lack of moisture and no contact of roots to the soil surface, death occurs to the formed roots. In the case of the exposure of this area to moisture for another time, new groups of roots will grow under the dead layer of roots resulting in pushing the dead roots causing their separation from the trunk. Repeated death of root groups and the growth of the other new groups from the inside will push dead roots until they separate from the trunk (Maki et al., 1998). So, each time the perimeter of the trunk at the separation area of leaf bases reduces and corrosion occurs in this area leading to a scraggy trunk, and thus a weak palm

The results of the investigation of leaf bases samples taken from the infected rot deformed areas on the trunks which were isolated, purified and defined showed the presence of the following fungi species: *Fusarium oxysporum*, *Fusarium solani* and Macrophomina *phaseolina*. It was observed that growth of the various fungi species on the deformed areas of leaf bases resulted from excess moisture which leads to rot occurrence and exposure to decomposition, causing the separation of the leaf bases from the trunk (El-Meleigi et al., 1993; Rashed and Abd El-Hafeez, 2001).

CONCLUSIONS

The case of apparent deformation on the trunks of date palm trees is a result from excess moisture on the leaf bases area because of water reaching directly to the trunk, leading the lateral roots to grow strongly under the leaf bases. Then they push the leaf bases causing their separation from the trunk. Deformation occurs also as a result from drought of the roots which were grown at the leaf bases area, and the continuity of exposing the trunk to periods of high moisture and drought leading to the growth of the roots. The drought of roots and their drops out of the trunk lead to corrosion and a scraggy trunk. The increase of moisture creates an appropriate environment which helps the growth of different fungi species on the trunk causing rot of the leaf bases and facilitating the separation of the leaf bases on the trunk. The reason for water reaching directly to the trunk is because of the damage caused by the irrigation system used, especially the bubbler irrigation method. In some cases, as a result of this damage and water reaching to the trunks, deformation occurs at various heights from the soil surface. Also the deformation occurs when using hand irrigation (by water hoses) and the water spray reaches directly to the trunk through the water outlets fixed in median islands of the streets or by irrigation with water mobile tanks. The results show the importance of using appropriate methods in irrigating the date palm trees and the need for following-up and running properly so that the water spray does not reach directly to the date palm trunks. Care should be taken of maintenance of irrigation systems used for the date palm trees planted in the streets in various districts in the Riyadh region. The irrigation systems should be designed in a way that water never comes into contact with the trunks of date palm trees especially when using spray irrigation for turfgrasses planted on the street median islands at the same location with date palm trees. It is advised to avoid the conventional irrigation (water hoses) where water reaches directly to the trunks of date palm trees, keeping them away from the exposure to moisture and preventing the separation of leaf bases from the trunk and the occurrence of the deformation.

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Tables

Table 1. Case of trunk's deformation of date palm trees planted in streets of the various districts in the Riyadh region.

Districts	Deformed trunks (%)	Length of deformed area (cm)			Height of deformed area from the soil surface (cm)			Length of the palm trunk (m)	Deformation (%)	Observation on Irrigation methods
		lowest value	highest value	Mean	lowest value	highest value	Mean			
King Fahad	100	25	121	66.73c*	0	169	5.52b	6.79d	9.93bc	Flood irrigation +
Al-Gamaa	100	15	214	81.43b	0	236	21.62a	7.16c	11.48b	Bubbler irrigation +
Al-Khozama	70.3	0	103	28.65d	0	1.00	0.4b	4.64e	6.26d	Drip irrigation +
Om Al-Hamam	100	49	217	119.62a	0	173	5.73b	8.58a	14.17a	Bubbler irrigation +
Al-Nasseriah	100	31	164	70.80bc	0	1.00	0.4b	8.11b	8.74c	Bubbler irrigation +
LSD at 0.05				12.53			12.62	0.21	1.83	

* Means followed by the same letter (s) within column are not significantly different at the 5% level of L.S.D. test.
+ Additional irrigation applied by mobile water tanks at water cease or by hoses connected to the fixed water outlets in median street islands.

Figures

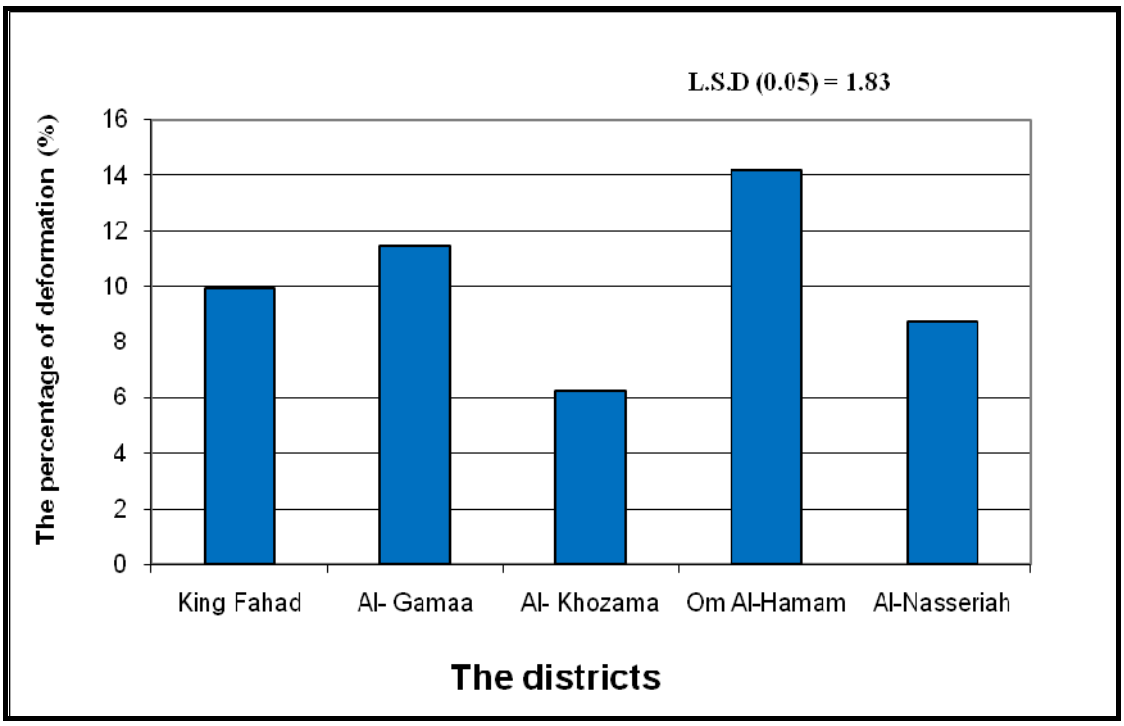

Fig. 1. The percentage of deformation (length of the deformation area/length of the trunk) of date palms grown in streets of the various Riyadh districts.

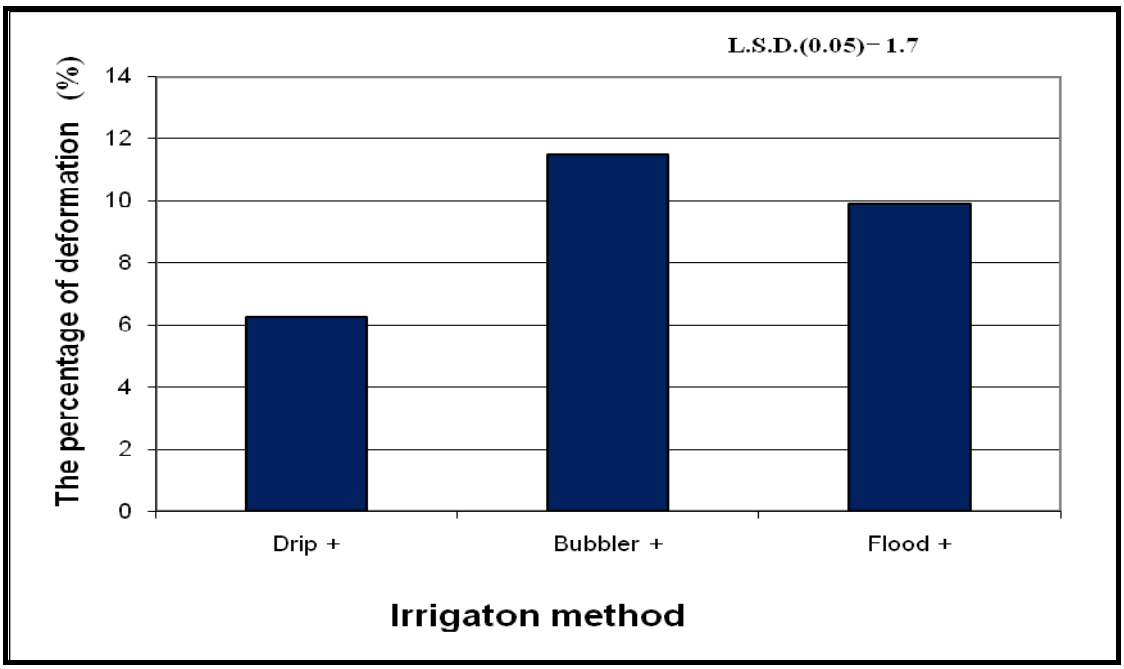

Fig. 2. The percentage of deformation (length of deformation area/ length of trunk) according to the observed irrigation method used for date palms. $^{+}$Additional irrigation applied by mobile water tanks at water cease.

Fig. 3. The beginning of the deformed area (separation of leaf bases) at the soil surface.

Fig. 4. Two deformed areas on the trunk, first at the soil surface and the other one up the trunk.

Worldwide Dispersal of the Date Palm from Its Homeland

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Keywords: geography, history, introduction, offshoots, *Phoenix dactylifera*, seeds

Abstract

Domesticated more than 5,000 years ago in Mesopotamia, present-day Iraq, the date palm (*Phoenix dactylifera*) is today a major world fruit crop in its original homeland, as well as in a number of other countries where it has been introduced for commercial production. Worldwide, date fruit production stands at about 6.8 million mt per year. Ten countries in the Middle East and North Africa account for 90% of the world's production. The remainder originates from some two dozen other countries including Australia, Mexico, Namibia, Peru, South Africa, Spain and the USA. In some of these other countries the date palm has not attained significant commercial status. Climatic, biological or economic factors are given as the general reasons for the lack of success, but the precise causes in each location have not been investigated in detail. In most instances, the date palm has become naturalized where it was introduced and has produced new undescribed seed-derived forms. In such circumstances the palms yield fruit for local consumption and are used as ornaments and play a role in the landscape. Over millennia, from Mesopotamia, the date palm has been carried in all directions in an historical process of diaspora that continues today. Where the date palm has been introduced, new local names have often been applied to known cultivars as well as to locally-derived seedling forms. A detailed overview of the dispersal of the date palm does not exist. In some instances, introducing the date palm to new areas provided an environment free of traditional pests and diseases of the palm; in other cases inadvertent pest and disease introductions have occurred along with date offshoots. This study summarizes the current state of knowledge regarding the diaspora of the date palm and identifies key areas where additional information is needed.

INTRODUCTION

The origin and dispersal of the date palm (*Phoenix dactylifera* L.) is a fascinating topic because it involves information from the disciplines of agriculture, botany, economics, geography, history and religion. A survey of the date palm literature was carried out to draw together the published accounts; especially references containing cartographic representations of the historical events and geographic patterns of dispersal.

The literature contains a number of distribution maps showing the countries where dates have been introduced and are currently found, but they lack historic or geographic background.

Considered to be among the oldest domesticated fruit tree crops, the date palm was part of the food production in the Near East at least 5,500 to 6,500 years ago, during the Chalcolithic period. Cultivated date palms appear to have been derived from wild and feral populations in warmer areas of the Near East, northern portions of the Arabian Peninsula and the northeastern Sahara Desert. Larger fruit size and much better quality mesocarp pulp distinguish domesticated from the wild forms of date palm. A map of wild and feral date palms in Zohary and Hopf (2000) shows a current distribution extending east to west from Mauritania to Pakistan and north to south from the Mediterranean Sea to northern Somalia. The combination of wild and feral populations obscures the important role of human dispersal of date palms within the broadly-defined region, whereby earlier

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human introductions have resulted in present-day feral populations. It should be kept in mind that date palms introduced to areas of suitable climate outside the above region have become naturalized and in some cases form contemporary feral populations, such as those found in Peru and Australia.

Apparently Fischer (1881) was the first to make a detailed study of the geography and spread of date palm cultivation, and he provided a map which depicts the introductions from place to place within the region delineated in the Zohary and Hopf map, as well as beyond: north into Europe and east to India.

Nearly a century later, Munier (1973), in his general book on date cultivation, included a modern distribution map of all 12 then recognized species of *Phoenix*; it portrays the overlapping distribution of some species which can result in natural hybridization. A recent comprehensive study of *Phoenix* systematics by Barrow (1998) sorts out the true identity of the species shown in Munier's map. In another map, Munier depicts the dispersal of date palm in two distinct patterns. One begins in Mesopotamia, and extends south into the Arabian Peninsula and east to Pakistan and India. The second starts from Egypt and spreads west across North Africa and into the Sahelian countries, and also south into Sudan.

Zaid (2002), the current standard reference book on date growing, contains a map reflecting the historical dispersal of the date palm and its cultivation for fruit, which is similar to and apparently based upon Munier. It includes date palm dispersal to the New World and Australia, but with very few details.

The purpose of this study is to summarize the current information regarding the diaspora of the date palm and its cultivation as it has occurred in historic times, beyond the broad west to east expanse from Mauritania to India. Specifically, this paper will focus to a major extent upon Europe and the Americas; to a lesser extent on southern Africa and the South Pacific.

Apart from the more academic aspects of this paper, there are three practical applications of the results. One, to help track the spread of date palm pests and diseases where these have been linked to the introduction of date palm offshoots and possibly fruits. Two, to assist in determining the origin of introduced cultivars, to identify synonymous local names permitting a better assessment of overall date palm germplasm resources. Three, to identify new genotypes that have emerged spontaneously in distant locations through sexual reproduction which may have desirable agronomic or commercial traits that could contribute to plant improvement for diseases and insect resistance, climatic regimes, productivity and so on.

In the discussion that follows, a clear distinction will be maintained between the geographic dispersal of dates by seed, which is the oldest and most prevalent means; the progeny commonly are designated as 'seedling dates'. Seedling date populations are composed of male and female palms; they may or may not be feral, but feral dates are always seed-derived. In contrast, there is the more modern practice of transporting offshoots, almost exclusively female, removed from highly desirable named date fruit cultivars, with the cultivar name typically being carried to the new location. In recent decades, date palms have been tissue cultured and plantlets shipped to various locations. That form of dispersal is not considered in this paper, although mention is made of their current role in some places. Also, excluded are introductions of date palms exclusively into botanic gardens or formal germplasm collections.

A final introductory comment needs to be made. This study is a preliminary effort which it is hoped will serve as a starting point for more detailed investigations into this broad and complex subject.

EUROPE

Spain

Historical accounts indicate that date palms were introduced by seed of unknown origin to Spain in the Christian era. However, they appear to have been of little

importance until the Moorish invasion and colonization beginning in the eighth century, which brought to Iberia knowledge of date culture and very likely new seed stocks. Elche, in Valencia just a few kilometers inland from the Mediterranean, was founded in the tenth or eleventh century. The Moors established an oasis agricultural system, modeled after the oasis system they knew, which included seedling date palms as a major component of the crop complex. Although at the northern limit for date fruit ripening (38°15'N. lat.), the palms provided fruit for local consumption, which continues today. Also, the trees are used in urban landscaping and as a source of blanched palm leaves prepared for the Palm Sunday procession which is a longstanding tradition in Elche (Ferry and Greiner, 1999; Gómez and Ferry, 1999).

Elche represents the only location in Europe where commercial date growing occurs, and currently has a population of about 150,000 seedling date palms. Offshoot propagation is not a local practice. Elche was designated a World Heritage Site in 2000 because of the historic date palm population (Ferry et al., 2002).

A new effort to expand commercial date production based upon local seedling genotypes is under development in Elche. The seedling date fruits exhibit an attractive variety of flavors, shapes, sizes and colors. The most promising local genotype is the 'Confitera'. The fruits are being promoted as fresh products to gourmet and organic food markets in Europe, some sold in rutab stage (Orts and Johnson, in press).

When Spain's exploration of the New World began in 1492, Elche and neighboring Orihuela were important cultural and religious centers with large date palm populations. They may well have a source of date palm seeds which were among the new plant species carried by the Spanish missionaries, who, in addition to spreading Christianity, played a key role in agriculture.

The Canary Islands also figure in the westward dispersal of date palm. Evidence suggests that the date palm was introduced in the fifteenth century during the European conquest of the islands. The origin of date seed was most likely the Iberian Peninsula, but possibly North Africa. At any rate, the date palms were well established in the islands when Spanish ships, beginning in the late fifteenth century, took on final provisions in the Canary Islands before crossing the Atlantic Ocean (Santana and Rodríguez, 1999).

Italy and France

Historical records could not be found to document the initial introduction of date palm to Italy and France; possibly seeds came from Spain. Any introduction would not have been for fruit production because the climate is unsuitable. It is known that since at least the sixteenth century, Bordighera, on the Italian Mediterranean coast near the French border, has been a source of blanched palm leaves for Christian Palm Sunday ceremonies. At present, the date palm population of Bordighera is in decline because of neglect; about 1,500 palms are left. When the Italian and French Rivieras were developed in the nineteenth century, dates and other palms were planted widely in gardens, parks and along roadways for ornamental purposes; the date palm populations of Bordighera were a source of ornamental planting material (Pintaud, 2002).

Red Palm Weevil

The following modern case study exemplifies the repercussions of accidental spread of an insect pest through international shipment of mature date palms.

Over the centuries of date palm cultivation in Spain and farther east in Europe along the Mediterranean Sea, there were no serious associated pest or disease problems. Originating in southeastern Asia as a pest on coconut, red palm weevil (*Rhynchophorus ferrugineus*) was spread westward by some means into the Middle East in the mid 1980s and from there dispersed within that region and into North Africa through the transport of infested date palm trees and offshoots. In the early 1990s it was found in Egypt, a time period which coincided with the new practice to import mature date palms from that country into Southern Europe for ornamental use. By the mid 1990s the weevil was found in the south of Spain, and later in other countries bordering the Mediterranean.

The insect is a serious threat to *Phoenix* palms in Europe (and elsewhere) because it appears not to have any local natural controls to its population. Detection of the pest is difficult and generally its presence evident only when a tree is seriously infected and beyond treatment. An ambitious research effort is under way focused on early detection and either biological or chemical control to manage this serious insect pest which has killed numerous *Phoenix* palms around the Mediterranean rim and is of particular concern to Elche, because the very identity of the city is linked to the presence of date palms (Ferry and Gómez, 2002).

THE AMERICAS

None of the species of *Phoenix* palms are native to the Americas. It is well documented that Spanish explorers were the first to introduce the date palm.

Caribbean and Mexico

Historical records place the date palm in Cuba, Hispaniola and Jamaica as of 1526; in Central Mexico in 1535. Spain is given as the origin but precise locations are not named, nor is it clearly stated that seeds were the means, but that is presumed to be the case. None of the above locations have climates suitable for fruit production, but some number of seedling date palms may have survived (Rivera et al., in prep.).

It was not until 1605 that date palms were reported in Sonora, northwestern Mexico, where growing conditions are favorable to the crop. However, for some reasons there were never any plantations in the state. I made a field survey of Sonora in 2004 which revealed only occasional seedling dates on farms and planted as ornamental trees in towns and cities. A park in the city of Hermosillo currently has an organized planting of date palms, but this is a recent development.

Spanish Jesuit missionary settlers in Baja California introduced dates to the peninsula in the middle of the 18th century where it also grew well. In time there came to be thousands of date palms at the major oasis locations of Loreto, Mulegé, San Ignacio and Comondú. Date palms in these four locations, and a number of smaller places, became naturalized in the landscape and have persisted through natural regeneration to the present day. Some fruit is harvested and marketed locally, but the trees receive no care. Many local people are surprised to learn that the date palm is not native.

Sonora is the obvious source of the date palm seed carried to the Baja Peninsula, but no documentation to support this has been found. Jesuit missionaries may have made new seed introductions directly from Spain, from North Africa, or even Peru (see below) where the Jesuit Order was active at that time.

In the latter half of the eighteenth century, the Spanish established a chain of missions extending northward in the peninsula. Date palm was a component of the European garden agriculture to support these missions.

Research is being conducted on the seedling date palms in Baja California to describe their botanical characteristics and, through DNA analysis, to try to ascertain the probable origin of the date seeds originally planted.

Peru and Chile

Date seed, said to be from the Barbary Coast of Africa (i.e., Morocco to Libya), were carried to the Central Coast of Peru in the early seventeenth century. It is not stated which route the seeds followed to reach Peru. The Jesuit scholar Bernabé Cobo (1964) observed ripe date fruits on the floodplain of the Pisco Valley in 1612. During the Spanish Colonial Period, the palms were a source of leaves for the pageants of Easter week. Seedling dates were dispersed to oases along the Peruvian Coast and formed naturalized sometimes feral populations. Eventually the dispersal reached what is now northern Chile. Because of the Colonial era trade links between Peru and Mexico, it has been postulated by Aschmann (1957) that Peru could have been a source of seed for the Jesuits who initiated date palm growing in the Baja California Peninsula.

The Atacama Desert has favorable climatic conditions for commercial date

plantations. Currently, there are estimated to be 50,000-80,000 mature female seedling date palms in Peru. At least ten of the local genotypes have been given local names, the most prominent being Calvario. Commercial date fruit production in Peru amounts to only a few hundred metric tons per annum; in Chile about 20 mt. Importation of date fruits is almost nil. Research on local genotypes has revealed some very promising characteristics. For example, a genotype which bears fruits weighing 42-45 g, and another which flowers and fruits continuously. Potential exists for either Peru or Chile to become a major supplier of date fruit for markets that could be developed in South America, and off-season exports to the Northern Hemisphere (Pavez et al., 2007).

The United States

A small number of seedling date palms were already being cultivated at Spanish missions and probably elsewhere, when California became part of the United States in 1848. The initial introduction is documented to have been in San Diego in 1769, from Baja California. In the second half of the nineteenth century, seedling dates were promoted as a crop because of the suitable climatic conditions in the interior of the state; some experimental research was done in California and Arizona, but this did not lead to an industry. Beginning in the 1890s and carried over into the early decades of the twentieth century, offshoots of named cultivars were imported from the Middle East and North Africa, through federal Government and private initiatives, and those offshoots created the basis for the small but successful date industry in the two states today. Irrigation canals and pumped groundwater sustain the artificial oases.

At present, the US date industry is not experiencing any major pest or disease problems, in part because of strict plant quarantine regulations which prohibit the transportation of any palm species into the date growing areas. An early problem did occur in the 1920s, when *Parlatoria* scale was brought in with offshoots, but it was eradicated through a rigorous phytosanitary program, which included treating infested trees and destruction of most seedling date palms, considered potential host plants. Bayoud disease has never been detected in the US but is of serious concern given the significant plantings of the highly susceptible 'Medjool' cultivar. Details of the various date introductions and development of the industry can be found in Hodel and Johnson (2007), Johnson and Hodel (2007) and Johnson et al. (2002).

Primarily because of the absence of major pests and diseases, California and Arizona are a highly desirable source of offshoots. In the past few decades I am aware of offshoots having been exported for commercial and experimental purposes to Mexico, Chile, Brazil, Spain, Israel, Namibia and Australia. This exemplifies the ongoing date palm dispersal at the cultivar level in the form of offshoots.

SOUTHERN AFRICA

Republic of South Africa

It has been estimated that the initial date seed plantings occurred in the country about a century ago; the origin of seed is unknown. At the middle of the current decade, there were more than ten commercial date palm growers in the Northern Cape Region; the farmers having obtained 'Medjool' and 'Barhee' offshoots (source not given) and are expanding cultivation with their own offshoots. Some small-scale plantings using 'Medjool' tissue culture plants also are reported. The area has few disease problems. A second date growing region is in Limpopo. However, climatic conditions there are not entirely suitable and moreover the African palm weevil and black scorch disease are both problems (McCubbin, 2007).

Namibia

This Southwest African country has climate conditions suitable for commercial date palm fruit production. Seedling dates have been growing along stream courses in Namibia since early in the twentieth century, possibly planted first by German settlers.

Popenoe (1973), writing in 1924, states that attempts were being made to establish commercial date fruit production in Damaraland, in the northwest Namib Desert. Neither the origin nor how date palm seed reached the country is documented.

In 1995 an ambitious date palm development program was launched with technical assistance from FAO (NDC 2000). A gene bank consisting of 24 cultivars and six clones were planted and a tissue culture laboratory established to provide planting material. In about 2002, an FAO news report stated that 10,000 date palms had been field planted in Nambia at Naute, Eersbegin and Aussenkehr in the southern part of the country. As of 2005 it was reported that abnormalities were being found in the maturing plants (INRA, 2005). The current status of the date palm program in Namibia is unclear.

SOUTH PACIFIC

Australia

The earliest date palm plantings in Australia were from seed, of unknown origin, in the 1880s at several locations in the Northern Territories and South Australia. In the 1890s, offshoots from Algeria were brought in, especially of the 'Deglet Noor' cultivar. None of the sites of early plantings any longer exist or have ceased fruit production. But, they did provide a seed source for the spread of date palms to stream valleys and springs over a wide area, to the extent that seedling date palm populations are considered now to be an invasive species threatening the native flora.

In the latter half of the twentieth century, additional imports of offshoots from named cultivars came in from California but losses were significant during a required one-year period of quarantine. More recently, tissue cultured plantlets have been imported. Date palms have been planted in Western Australia, as well.

The Mecca Date Garden, located 70 km southeast of Alice Springs, is the largest commercial date producer in the country. Fruit of the 'Medjool', 'Deglet Noor', 'Barhee' and 'Kalas' cultivars are produced. This date farm has made a collection of at least 14 interesting seedling date palm genotypes, by transplanting mature trees or offshoots from various locations. These represent a potential germplasm resource for research purposes.

Central Australia's arid climate is suitable for date fruit production; the key drawback to large-scale cultivation being an insufficient supply of water. *Parlatoria* scale, inadvertently introduced, is the major pest problem but could be controlled. It remains to be seen what the future holds for the date fruit industry in Australia. This account is drawn from Carpenter (1988) and Tanswell (1999).

New Caledonia

An interesting detail of date palm history involves this island of the South Pacific, the most remote location of the palm's dispersal. In the period of about 1864-1894 insurgents from the French colonies of North Africa, especially Algeria, were deported to the French colony of New Caledonia. Deportees carried with them date palm seeds, which were planted at the rural penitentiary in the region of Bourail, where the climate is semi-arid. The palms were planted because the fruit represented a staple food of the prisoners, but never became very important. As the deportee generation died off, interest in the crop waned. However, seedling date palms continue as a part of the culture and landscape of Bourail (Ouennought and Kahn, 2005).

CONCLUSIONS

Date palm has played a unique role in human history. From its initial domestication in Mesopotamia it has been dispersed by means of complex patterns associated with European exploration and colonization, religion, agriculture and modern commerce. Date palm has been called the "tree of life" because of its multiple utility; it could also be designated as the "tree of history".

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Fruit Characterization of Present Cultivars and Some Newly-Found Genotypes of Date Palm in Hormozgan Province of Iran

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Abstract

So far, more than 400 date cultivars have been recognized in Iran. When introduced to different regions of Iran, these cultivars significantly produced different quality from those in the area of origin. Ongoing studies deal with comprehensive characterization of these cultivars and identifying more cultivars. The present study was carried out to identify more new genotypes for identity certification and to select the cultivars which best suit the packaging and processing industries. The experiment was conducted with more than 100 cultivars from different regions using a sample of 200 fruits for each cultivar. Of these groups, the number of dominant cultivars throughout the regions of Siahoo, Minab, Rudan, Hadji-Abad, Banda-e-Lengeh, Bastak, Parsian, and Fin-o-Rezvan were 17, 12, 26, 20, 14, 23, 21 and 39, respectively. Fruits were tested for some quality criteria using both chemical and physical analyses. Chemical characteristics were pH, acidity, TSS, reducing sugars, total sugar, moisture content percentage and dry matter percentage. Physical characteristics were fruit length, diameter and weight; seed length, diameter and weight; pulp weight; pulp/seed ratio. The most important commercial cultivars for packaging found to be the soft-fruit cultivars 'Khunazi', 'Mordaseng', 'Khasab', 'Khallas', 'Barhee', 'Negar', 'Hallawi', 'Lasht', 'Ghorbani', 'Mozafati', 'Shahani', 'Nohdanehgazi', 'Khasouei', 'Istaemran', 'Mosalli', 'Kabkab', 'Breim', 'Zamardan', 'Kariteh' and 'Nabati' and 5 semi-dry cultivars 'Piyarom', 'Dayri', 'Medjool', 'Zahidi' and 'Thoory'. The rest of the cultivars were found suitable for the processing industry. The cultivars 'Nohdaneh Gazi' with golden yellow color, long fruits with least fiber and 'Hassan Mahdi' a shiny soft date, resistant to fermentation with dark brown color, and least fiber and 'Moghbali Modadal' a soft date with soft tissue and light brown color were added as new genotypes to the Iranian date germplasm.

INTRODUCTION

Hormozgan is a province in the southern central region of Iran (25°44" to 28°58"N;52°41" to 59°15"E) with a total land area of 68,418 km^2 which is bordered along the south by the Persian Gulf and the Oman Sea. There is a significant variation in climate across the province. Low rainfall, dry-hot summers and cold winters are typical for the inland north and low rainfall and humid-warm summers with temperate winters occur along the coastal strip. Hormozgan is divided into 11 officialy recognized regions of Abu-Mousa, Bastak, Bandare-e Khamir, Bandar-e-Abbass, Bandare-e-Lengeh Jask, Hadji-Abad, Rudan, Qeshm, Parsian and Minab containing 29 division and 76 rural districts each one having a microclimate of arid to semi arid regions. With a total annual production of 157,000 tons date and cultivation areas of 39375 ha, Hormozagan province is the country's largest date producer in terms of both production and area. Of the total production 34% is served as fresh or packed date, 51% goes to the processing industries and the remaining 15% is waste due to poor quality. More than 100 cultivars are grown in this province. Some are well known cultivars in the world while some are just cultivated in small regions and have never been introduced to other areas or even studied. Cultivars

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perform differently under different climatic conditions of this province. The majority of date cultivars are soft. Of 49 date processing units under construction only 11 units are operative with a total capacity of 9258 tons. The main products of this industry are packed date, date juice, sauce, ethanol, date sugar, date honey, date paste and date vinegar. These units are planned to be thoroughly supplied by the province's produces.

With such a rich date germplasm supplying the industry it would be very easy if all cultivars in this province are precisely characterized. Moreover, it is generally accepted that the chemical composition of date fruit and its shape, size and weight vary with the cultivar and climatic condition. Little work has been done in Hormozgan to characterize the fruits of cultivars under climatologically different regions and even on some cultivars at all. Recently there have been some efforts to make a comprehensive atlas of Iranian date palm cultivars which comprises more than 400 cultivars (FAO, 1996) of unknown and well known characteristics. Davoodian (1989) grouped Hormozgan date in terms of quality to first, second and third rate cultivars. The cultivars 'Istaemran', 'Piyarom', 'Zahidi', 'Barhee', 'Mordaseng', 'Hallawi', 'Khasouei', 'Kabkab', 'Shahani', 'Dayri' and 'Mozafati' have been named as the most commercial cultivars of Iran (Pejman, 2001). No comprehensive characterization has been carried out for date cultivars of Hormozgan. However, these cultivars are scattered in different regions which perhaps produce different qualities.

Researchers in date producing countries have studied qualitative traits of date cultivars (Al-jasim and Al-Delaimy, 1942; Knight, 1960; Peopnoe, 1973; Seeling, 1978; Morton and Julia, 1982; Yektankhodaee, 2002). Studies have been carried out to measure the nutritional composition of date palm (Yousif et al., 1998), organics and inorganic constituents of date palm (Elshurafa et al., 1962), pectin contents (Hussein et al.), organic acids (Rygg, 1952), enzymes involving fruit ripening (Hasegawa et al., 1962). In a study in which some date cultivars were assessed for moisture content, reducing sugar and total sugar, 'Zahidi' showed to be a dry cultivar with high yield, 'Khadrawi' had a delicious taste and 'Halawi' a light color and soft tissue (Cook and Furr, 1952). Most of such chemicals are influential in date quality and dependant on cultivar, climate conditions and agricultural practices. Yousif et al. (1998) evaluated 4 cultivars of Iraq and stated that the glucose/fructose ratio is dependant on climatic conditions. Supposing the agricultural practices could be optimized still climate and cultivars remain influential. The effect of such single factors and interaction between them must be determined via evaluation of date fruit quality. Therefore, this study was aimed at:

- Introducing suitable date cultivars to the industries as a strategy to support both industry and farmers through the characterization of all cultivars of the province in terms of physio-chemical characteristics which are highly dependent on climate.
- Reporting new cultivars present in Iran germplasm and used locally.
- Contributing to the preparation of a national atlas of date cultivars to share these data with fellow date researchers all over the world.

MATERIALS AND METHODS

Well known and locally known cultivars of date from different regions of the province were evaluated for physio-chemical characteristics and classified into three qualitative groups of first, second and third class. Two hundred fruits were harvested from each tree at horticultural maturity at three recognized ripening stages of date fruit; khalal, rutab and tamar and the following tests were conducted:

- Appearance: a visual assessment was made on the basis of shape, color, freshness percentages of defects or blemishes and fruits were graded.
- Total soluble solid (TSS) was measured with a refractometer from extracted juice from 25 g date fruit mixed in 100 ml distilled water.
- Reductive and total sugar was measured according to the Lin-ainon (Ranganna, 2000). method adjacent to methylene marker.
- The percentage of dry matter (dry weight/fresh weight × 100) and moisture content were determined using 10 g of fruit pulp in a petri dish in an oven at 70-75°C for 72h.

- 10 fruits were tested for whole fruit length and diameter, seed length and diameter, fruit pulp and seed weight, fruit volume and pulp/seed ratio.

RESULTS

Twenty five cultivars were identified as first class including 20 soft tissue: 'Khunaizi', 'Mordaseng', 'Khasab', 'Khallas', 'Barhee', 'Negar', 'Hallawi', 'Lasht', 'Ghorbani', 'Mozafati', 'Shahani', 'Nohdanegazi', 'Khasouei', 'Istaemran', 'Mossalli', 'Kabkab', 'Breim', 'Zamardan', 'Karite', 'Nabati', and 5 dry cultivars 'Piyarom', 'Dayri', 'Medjool', 'Zahidi' and 'Thoory' (Tables 1-18). A large number of second class cultivars including 'Khoshkong', 'Nimakdini', 'Khorusi', and third class cultivars were identified (Tables 1-18). The most important commercial cultivars for packaging were found to be soft-fruit cultivars 'Khunazi', 'Mordaseng', 'Khasab', 'Khallas', 'Barhee', 'Negar', 'Hallawi', 'Lasht', 'Ghorbani', 'Mozafati', 'Shahani', 'Nohdanehgazi', 'Khasouei', 'Istaemran', 'Mosalli', 'Kabkab', 'Breim', 'Zamardan', 'Karate' and 'Nabati' and 5 semi dry cultivars 'Piyarom', 'Dayri', 'Medjool', 'Zahidi' and 'Thoory', the rest of cultivars were found suitable for the processing industry. The cultivar 'Nohdaneh Gazi' (found in Hajiabd) with a golden yellow color, long fruits with least fiber and 'Hassan Mahdi' (found in Fin-o-Rezvan) a shiny soft date, resistant to fermentation with dark brown color, and least fiber and 'Moghbali Modadal' a soft date with soft tissue, light brown were added as new genotypes to the Iranian date germplasm (Fig. 1). The classification of date fruit in each regions is detailed as follows:

Minab Region (Hasht-Bandi, Karyan, Goorband)

Some physio-chemical characteristics of fruits of cultivars grown in this region are shown in Tables 1 and 2. Superior cultivars in these regions were 'Nabati', 'Mordaseng', 'Khunazi', 'Khasab', 'Karite', 'Hallawi', 'Negar', 'Deyri', 'Almehtary', 'Barhee', 'Raana-tala' ('Khalas'), 'Abu Narenja'. One of the first class such as 'Almehtari', is an early ripening cultivar in this region and resistant to relative humidity at the time of ripening and is presented early in the market for fresh edible consumption. 'Hallawi', 'Deyri' and 'Raana-tala' are among cultivars which are resistant to fermentation and decay caused by high RH. Date fruit of 'Khunazi' and 'Barhee' at the stage of khalal are sweet and often served fresh. The characteristics of the second and third class cultivars are presented in Tables 1 and 2.

Rudan Region (Ziaratali, Jaghin)

Some physio-chemical characteristics of fruits of cultivars grown in this region are shown in Tables 2 and 3. Of the first class cultivars in this region are 'Farz', 'Mozafati', 'Kariteh', 'Mordaseng', 'Azar', 'Lasht', 'Khuneizi', 'Kabkab' and 'Negar'. 'Negar' is of the first class and suitable for export. To avoid date fermentation at the stage of tamar on trees, fruits must be harvested at the stage of rutab. The characteristics of the second and third class cultivars are presented in Tables 3 and 4.

Hadjiabad Region (Tezerj, Sirmand, Ahmadi)

This high inland region is far from the sea and enjoys a relatively dry climate where tamar fermentation does not occur on trees. Existing cultivars in this region are superior compared to those grown in the humid costal strip. 'Piyarom', 'Khasouei', 'Mozafati', 'Dayri', 'Mordaseng', 'Kabkab', 'Hallawi', 'Thoory', 'Barhee', 'Negar', 'Zahedi', 'Nohdaneh Gazi', 'Khuneizi', 'Lasht', 'Medjool', 'Kariteh', 'Shahani', 'Marzeban'. The fruits of 'Piyarom' and 'Zahidi' must be harvested in semi dry stage and the fruit of 'Dayri' and 'Thoory' after drying. The characteristics of the second and third class cultivars are detailed in Tables 5 and 6.

Parsian Region

This area is located in the coastal line with a warm humid climate. Date cultivars in this area are subjected to fermentation and decay in the rutab stage. Among the first

class cultivars in this region are ‘Moosali’, ‘Khasooei’, ‘Azad’, ‘Estameran’, ‘Zahedi’, ‘Marzebane Kivar’, ‘Zamardan’, ‘Khuneizi’, ‘Marzeban Daryaei’, ‘Kabkab’, ‘Beraimi’, ‘Mordaseng’, ‘Maktoom’, ‘Khazrawi’, ‘Ghasbe’, ‘Marzeban’. ‘Breim’ is one of the sweet unripe fruit cultivars. ‘Azar’, ‘Kabkab’ and ‘Mosalli’ are export cultivars. Second class cultivars are ‘Hameron’, ‘Shahri’, ‘Khosh kong’, ‘Fiba’, ‘Michotonbaky’, ‘Narmchahi’, ‘Khoravifaryab’, ‘Haji Mohamadi’, ‘Piyarom’, ‘Koshkharak’, ‘Peypa’, ‘Kach Kha’, ‘Mazrooei’, ‘Maleson’, ‘Rajonehei’ with soft tissue and no fiber. Third class cultivars are ‘Halo’ which is not suitable for packing. The characteristics of the second and third class cultivars are presented in Tables 7 and 8.

Bandare-e-Lengeh Region

The first class cultivars of this coastal region are ‘Khunazi’, ‘Zamardan’, ‘Ashghari’, ‘Moosali’, ‘Barhee’, ‘Mordaseng’, ‘Mozafati’, ‘Khasab’, ‘Shaham’, ‘Marzeban’ and ‘Azad’. ‘Shaham’ and ‘Kuneazi’ produce sweet tasty fruits at the stage of khalal. Second class cultivars are ‘Kharook poshte baghe’, ‘Khosravi’, ‘Sorkh Dang’, ‘Gentar Pibayejamil’, ‘Shahani’, ‘Kabkab’, ‘Shekarpare’, ‘Gajkhah’, ‘Khosh Kharak’, ‘Boldooz’, ‘Hallili’ with soft tissue and sensitivity to fermentation but suitable for drying and packing. Also ‘Ashghari’ and ‘Khosh Cong’ are harvested in khalal stage. The characteristics of the second and third class cultivars are presented in Tables 9 and 10.

Bastak Region

‘Lasht’, ‘Barhee’, ‘Marzeban’, ‘Azar’, ‘Mozafati’, ‘Khalas’, ‘Moosali’, ‘Khasooei’, ‘Mordaseng’, ‘Zardelar’, ‘Ryab Haji’, ‘Kuneizi’, ‘Beriem’, ‘Estameran’ are the first class cultivars in this region. ‘Khasouei’ and ‘Azar’ are export cultivars. ‘Khos Kharak’ has sweet unripe fruits at early khalal tage. Third class cultivars are ‘Khoravi’, ‘Karyab’, ‘Haji’, ‘Hallo’, ‘Kharook’, ‘Poshtebagh’, with soft tissue, and sensitive to fermentation. The characteristics of the second and third class cultivars are presented in Tables 11 and 12.

Fin-o-Rezvan Region

‘Lashti’, ‘Negar’, ‘Mozafati’, ‘Khasooei’, ‘Mangenas’, ‘Kuneizi’, ‘Marzeban’, ‘Hasanmahdi’, ‘Thoory’, ‘Zahedi Kariteh’, ‘Zohreei’, ‘Nabati’, ‘Moosali’, ‘Mordaseng’, ‘Khasab’ are among the first class cultivars in this region with red and yellow unripe fruit which are well known to local people. In this study these cultivar were added to the list of Iranian date palm germplasm. The characteristics of the second and third class cultivars are presented in Tables 13 and 14.

Siyahoo Region

This is a region receiving scattered showers at the time of fruit ripening causing fruit cracking and fermentation. ‘Farz’, ‘Mozafati’, ‘Khuneizi’, ‘Marzeban’, ‘Khasooei’ are among the first class cultivars in this region. The characteristics the of second and third class cultivars are presented in Tables 15 and 16.

Qeshem Region

This is an island in the Persian gulf with moderately high RH at all stages of fruit development and ripening. ‘Khasab’, ‘Mordaseng’, ‘Barhee’, ‘Marzeban’ are among the first class cultivars in this island. The characteristics of the second and third class cultivars are presented in Tables 17 and 18.

DISCUSSION

Harvested fruit at khalal stage for some cultivars like ‘Barhee’, ‘Khallas’, ‘Khosh-Kharak’, ‘Ashghari’ and ‘Breim’ are susceptible to fermentation and decay due to a moisture content of 50-85%. Fruits which are harvested at rutab stage have less moisture content about 30-45% which is only susceptible to fermentation. In the costal region with high RH from May through September, conversion of rutab to tamar will not proceed

properly and often fermentation occurs on trees. Such adverse conditions cause the first class dates to be considered as second class dates. At the stage of tamar, the moisture content of fruits falls below 25% and even in some dry regions drops under 10%. In some cases, to avoid any fermentation at the storing practices, once fruit is harvested additional dehydration by solar radiation or special dryer machines is needed.

It appears there are a wide range of produces for industries for all stages however some revision must be made in terms of establishing new orchards or substituting existing orchards. By conducting such a study and providing the industries with the table of physiochemical characteristics it is expected that they could pinpoint the produce they need for different purposes.

The results recommend to grow cultivars with high quality khalal and to some degree rutab in coastal regions and all old cultivars grown for tamar must be also replaced by such cultivars. Another strategy is to harvest rutab rather than tamar and store it at low temperature in small packages and distribute it to retailers or supermarkets. In far regions from the sea like Hadji-Abad however, the dry and soft dates such as 'Zahidi' and 'Piyarom' appear very desirable, the cultivars producing high quality tamar are also recommended. Some cultivars like 'Nohdanehgazi', 'Hassan Mahdi' and 'Moghbali Modadadl' have been added to Iranian date germplasm but they have yet to be identified and compared with other cultivars through molecular tools such as DNA markers.

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Tables

Table 1. Some chemical characteristics of fruits of date cultivars in different regions of Minab (Hasht-Bandi, Karyan, and Goorband).

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Nabati	6.97	0.10	42.20	46.14	58.46	2.95	44.40	55.60	
Karite	6.40	0.25	62.40	63.34	69.00	5.37	14.20	85.80	
Mordaseng	6.71	0.31	50.64	46.50	58.31	11.69	29.80	70.18	
Hallawi	7.20	0.10	46.80	47.50	50.67	3.01	39.90	60.10	
Barhee	7.59	0.08	46.20	46.10	48.26	2.05	35.05	35.20	
Ranatala	7.59	0.12	42.80	40.00	41.08	1.02	42.50	57.50	1
Almehtari	7.14	0.27	47.12	51.72	58.28	6.22	18.38	81.42	
Negar	6.58	0.16	44.00	27.14	46.06	17.97	16.90	83.10	
Marzban	7.25	0.11	44.80	42.23	42.23	0.00	32.60	67.50	
Abunarenja	6.58	0.14	50.40	54.28	54.28	0.00	32.50	67.50	
Deyri	6.63	0.30	54.40	56.29	56.29	0.00	24.60	75.40	
Khoshkong	6.69	0.16	43.60	35.18	47.50	11.69	32.05	67.95	
Nimakdini	7.54	0.14	34.00	44.70	47.50	2.66	41.60	58.40	
Danesefid	7.52	0.11	46.00	42.23	42.23	0.00	36.60	63.40	2
Khorusi	6.45	0.23	47.73	47.72	53.25	5.24	28.76	71.20	
Kabkab	6.79	0.15	47.80	46.86	48.26	1.33	28.70	71.30	
Shishie	5.43	0.33	56.60	69.09	69.09	0.00	11.10	88.90	
kharuksorkh	7.09	0.16	36.00	36.19	44.70	8.08	42.90	57.10	
Khro zard	7.25	0.08	30.00	44.70	47.50	2.66	36.60	63.40	
Kharuk Mordaseng	7.43	0.14	40.00	47.50	38.00	3.28	32.60	67.40	
Mozafati	6.92	0.16	49.06	49.06	49.64	0.55	35.80	64.20	
Zarek	6.84	0.18	47.28	47.28	49.83	2.41	29.82	70.18	
Kharuk	7.17	0.12	39.80	38.09	38.09	0.00	45.55	54.45	3
Kharuazari	7.24	0.08	34.80	44.70	40.00	-	43.60	56.40	
Zardun	5.75	0.38	64.00	63.34	69.09	5.46	12.40	87.60	
Piyarom	6.63	0.12	45.20	44.70	44.70	0.00	41.70	58.30	
Ghasab Shahdad	7.44	0.08	42.80	42.23	50.67	8.01	39.10	60.90	
Gardun	5.76	0.21	53.20	52.41	60.08	7.97	24.60	75.40	
Halo	5.18	0.26	42.20	47.38	48.23	0.80	41.00	73.10	

Table 2. Some phyical characteristics of fruits date cultivars in different regions of Minab (Hasht-Bandi, Karyan, and Goorband).

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Nabati	42.72	21.58	17.60	15.94	1.63	10.39	26.73	7.50	
Karite	36.09	22.08	8.56	10.04	0.90	11.16	21.72	6.30	
Mordaseng	26.01	19.33	8.32	7.62	0.72	10.31	19.13	6.78	
Hallawi	32.99	17.88	8.96	7.93	1.03	7.69	20.30	8.61	
Barhee	27.78	21.41	10.87	10.16	0.88	11.78	15.35	7.37	
Ranatala	37.23	23.03	16.31	15.41	0.89	17.31	21.99	8.24	1
Almehtari	32.21	17.24	6.90	6.04	0.83	7.43	21.96	6.60	
Negar	34.91	15.46	4.86	3.80	1.05	3.61	24.39	7.79	
Marzban	30.10	19.24	8.67	7.92	0.76	10.41	19.18	6.63	
Abunarenja	34.72	24.63	14.86	13.56	1.44	9.41	19.20	9.28	
Deyri	34.09	17.45	8.51	7.26	1.25	5.80	24.36	18.92	
Khoshkong	33.74	17.44	7.96	6.04	1.03	5.12	22.97	9.23	
Nimakdini	37.72	-	-	-	-	-	-	-	
Danesefid	27.52	17.00	5.51	4.73	0.75	6.25	18.47	6.63	
Khorusi	27.68	18.02	7.71	6.80	0.88	7.54	21.24	6.26	2
Kabkab	27.17	17.88	7.61	6.93	0.68	9.03	19.89	6.99	
Shishie	35.34	14.71	5.57	4.69	0.87	5.39	25.41	5.92	
Kharuksorkh	33.27	17.37	6.89	5.68	1.20	4.74	21.45	9.20	
Khro zard	27.90	16.90	6.97	6.05	0.87	6.95	19.02	7.60	
Kharuk Mordaseng	29.62	21.01	14.06	13.13	0.79	16.61	22.09	61.65	
Mozafati	29.77	19.95	10.44	9.64	0.79	11.96	18.76	7.86	
Zarek	32.42	16.27	6.54	5.65	0.89	6.51	21.98	7.42	
Kharuk	30.75	17.30	8.07	7.23	0.80	6.57	20.83	6.56	
Kharuazari	36.90	17.61	8.84	7.73	0.93	8.27	26.05	6.50	3
Zardun	38.08	15.87	5.64	4.93	0.71	6.94	23.14	6.07	
Piyarom	38.91	17.25	9.71	8.78	1.03	8.52	25.67	6.26	
Ghasa bshahdad	36.72	19.18	12.46	11.64	0.81	14.37	24.32	7.60	
Gardun	31.37	19.66	7.62	7.04	0.57	12.35	19.76	6.18	
Halo	37.91	22.28	12.83	11.95	0.85	13.96	21.99	6.97	

Table 3. Some chemical characteristics of fruits of date cultivars in different regions of Rudan (Ziaratali and Jaghin).

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Farz	6.70	0.16	51.80	51.96	57.96	9.10	29.46	53.70	
Mozafati	6.46	0.22	56.40	62.43	67.55	5.72	20.65	79.35	
Karite	6.90	0.20	53.15	62.19	65.05	4.79	23.88	76.11	
Mordaseng	6.70	0.20	58.64	63.19	57.70	4.78	27.80	71.28	
Azar	5.97	0.24	58.40	60.32	61.71	1.32	19.23	80.76	
Lasht	6.14	0.12	46.80	47.68	54.02	6.01	35.15	64.85	
Kheneizi	6.90	0.18	52.26	48.00	57.49	9.00	26.13	73.86	
Kabkab	6.49	0.16	52.40	63.34	63.34	0.00	28.40	71.60	
Nesfei	6.83	0.14	48.00	50.67	50.67	0.00	35.20	64.80	1
Azad	7.20	0.11	44.00	38.00	42.23	4.01	43.00	57.00	
Zohrei	7.14	0.11	48.00	47.50	47.50	0.00	30.00	70.00	
Moghbali	6.17	0.20	40.20	40.11	55.66	14.76	37.90	62.10	
Ghorbani	5.86	0.26	47.20	50.67	72.50	20.73	28.90	71.10	
Almehtari	7.02	0.25	50.00	49.08	50.67	1.50	12.90	87.10	
Shahani	6.35	0.24	56.93	59.11	65.83	6.37	21.20	78.80	
Khasui	7.38	0.12	54.00	54.28	54.28	0.00	19.60	80.40	
Negar	5.86	0.33	56.20	57.00	63.77	6.43	16.00	84.00	
Abdandan	6.70	0.16	52.00	54.28	58.46	3.97	30.10	69.90	
Bardial	6.50	0.15	51.40	53.97	56.51	2.41	26.10	73.90	
Khorusi	7.08	0.14	46.00	44.70	52.28	7.20	20.20	79.80	
Batahodi	6.42	0.11	48.00	47.50	50.67	3.01	35.00	8.65	
Lelengani	6.09	0.22	52.40	54.30	69.00	13/96	26.40	73.60	2
Berni	5.86	0.25	63.20	63.34	69.00	4.37	13.10	86.90	
Shekari	6.44	0.27	63.20	58.46	69.09	10.09	12.40	87.80	
suzun	7.52	0.11	45.60	47.50	47.50	0.00	32.20	67.80	
Angoshtarus	5.82	0.33	58.00	58.46	95.00	34.71	19.50	80.50	
Halo	5.18	0.19	36.00	36.10	38.00	1.80	35.90	64.10	3

Table 4. Some physical characteristics of fruits date cultivars in different regions of Rudan (Ziaratali and Jaghin).

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Farz	36.14	20.35	10.84	10.23	0.59	17.30	18.91	5.86	
Mozafati	35.21	22.53	11.12	12.16	0.93	13.00	21.53	6.73	
Karite	35.35	21.03	10.26	9.53	0.85	11.38	19.88	6.76	
Mordaseng	28.17	19.43	8.76	7.96	0.77	10.36	17.78	7.98	
Azar	42.87	18.03	8.56	7.58	0.95	7.80	26.35	6.27	
Lasht	39.28	21.53	13.12	12.25	0.83	15.59	23.95	6.48	
Kheneizi	34.51	20.47	13.30	9.36	0.67	13.83	20.23	6.56	
Kabkab	43.72	22.35	15.70	0.91	14.69	16.14	24.85	6.22	
Nesfei	41.72	24.84	16.27	15.49	0.78	19.85	17.40	8.30	1
Azad	48.60	21.00	13.77	1.34	12.43	9.27	29.43	7.97	
Zohrei	46.01	20.72	10.84	10.18	0.66	15.42	20.17	6.30	
Moghbali	41.75	20.88	15.60	14.59	0.97	15.01	21.45	6.61	
Ghorbani	37.27	21.01	13.06	12.10	0.86	13.87	20.42	6.67	
Almehtari	30.42	14.21	4.89	4.20	0.78	5.35	22.81	6.07	
Shahani	38.12	15.88	6.04	5.19	0.83	6.22	24.66	6.07	
Khasui	23.79	15.85	4.96	4.43	0.52	8.51	15.28	6.24	
Negar	36.92	15.62	7.90	6.71	1.17	5.73	25.36	7.41	
Abdandan	33.23	19.21	7.03	6.20	0.82	7.56	18.00	6.97	
Bardial	35.86	21.01	10.09	10.73	0.94	11.23	22.44	6.59	
Khorusi	32.36	17.02	7.37	6.59	0.76	8.67	17.99	6.90	
Batahadi	35.11	18.43	10.35	9.54	0.86	11.10	22.92	6.33	
Lelengani	35.40	18.33	10.61	9.66	0.89	10.74	23.15	6.05	2
Berni	34.61	17.64	7.54	6.78	0.74	9.11	20.93	6.23	
Shekari	31.42	18.60	7.43	6.44	0.99	6.40	21.15	7.64	
Suzun	35.85	17.32	9.31	8.40	0.90	9.34	21.85	6.22	
Angoshtarus	50.28	17.78	11.04	9.90	1.12	8.83	32.27	6.97	
Halo	34.35	21.95	11.80	10.90	0.85	12.82	22.21	7.54	3

Table 5. Some chemical characteristics of fruits of date cultivars in different regions of Hadji-Abad (Tezerj, Sirmand, and Ahmadi).

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Zahedi	5.94	0.25	63.10	64.77	69.09	4.09	12.72	87.22	
Nohdanegazi	6.94	0.19	48.00	54.28	54.28	0.00	23.20	76.80	
Kheneizi	7.28	0.13	49.28	50.61	54.05	3.26	30.78	69.22	
Khasui	6.68	0.20	32.59	59.65	62.51	2.71	21.47	78.53	
Lasht	6.08	0.28	58.08	56.78	58.36	1.50	22.02	77.98	
Medjul	5.97	0.34	61.40	23.42	69.67	43.93	15.53	84.46	
Marzban	6.55	0.20	55.73	56.09	57.49	1.46	19.00	81.00	
Shahani	6.42	0.22	55.91	58.00	60.23	2.11	22.99	75.07	
Mordaseng	6.38	0.20	53.60	50.89	50.89	0.00	31.76	68.23	1
Kabkab	6.52	0.24	54.80	57.33	59.34	1.91	23.70	76.30	
Halavi	6.21	0.27	63.00	67.94	72.46	4.29	11.30	88.70	
Deyri	6.08	0.26	64.10	70.81	71.64	0.78	11.32	88.67	
Piyarom	6.42	0.23	60.42	63.61	64.08	1.36	17.10	82.95	
Negar	5.96	0.35	61.90	62.98	64.49	1.43	13.82	86.17	
Turi	6.23	0.22	57.00	65.14	65.14	0.00	13.05	86.95	
Berhi	6.90	0.20	57.60	60.61	63.69	2.92	17.76	82.23	
Karite	7.47	0.14	44.80	47.50	50.67	3.01	39.20	60.80	
Mozafati	6.83	0.17	55.32	58.22	60.19	1.54	20.41	79.58	
Siahbizu	6.46	0.74	60.80	60.8	63.34	2.41	21.00	79.00	
Zard	6.78	0.16	54.00	54.28	54.28	0.00	25.30	74.70	
Asnaf	5.83	0.16	44.80	44.70	44.70	0.00	40.20	59.80	
Lelengani	6.25	0.14	41.80	44.86	47.68	2.67	42.75	57.25	
Shekari	6.19	0.22	61.60	69.09	69.09	0.00	16.40	83.60	
Kharkalut	7.06	0.13	45.60	47.54	49.08	1.45	31.90	68.10	
Almehtari	6.94	0.19	48.00	54.28	54.28	0.00	23.20	76.80	
Anbari	7.09	0.11	46.80	47.50	50.67	3.01	33.30	66.70	
Kharuzard	6.67	0.14	46.00	56.29	56. 29	0.00	36.60	63.40	
Zarek	6.38	0.15	41.60	56.29	56.29	0.00	42.60	57.40	
Torshuk	5.81	0.22	48.00	58.46	58.46	0.00	36.40	63.60	
Paselari	-	-	-	-	-	-	-	-	
Kalut	5.32	0.22	44.80	54.28	54.28	0.00	34.60	65.40	
Ferekan	6.69	0.18	54.72	52.02	55.45	3.26	16.18	83.82	2
Kharakpiarom	7.19	0.11	36.80	37.07	38.97	1.80	47.20	52.80	
Kharu	6.17	0.19	52.80	54.28	56.29	1.90	29.10	70.90	
Kharushahani	6.92	0.19	52.00	54.28	58.46	3.97	33.60	66.40	
Bedunenam1	7.01	0.11	46.00	50.67	54.28	3.42	33.80	66.20	
Melson	6.58	0.12	52.00	47.50	47.50	0.00	25.00	75.00	
Bardivar	7.09	0.11	46.80	47.50	50.67	3.01	33.30	66.70	
Kharakmozafati	7.25	0.10	32.80	33.04	38.000	4.71	56.00	44.00	
Khansiri	7.42	0.21	59.60	56.29	58.46	2.06	29.00	71.00	
Halili	6.68	0.16	48.80	50.67	54.37	3.51	27.30	72.70	
Bardial	6.90	0.16	51.80	50.89	53.74	2.71	22.70	77.30	
Pasmash	6.67	0.14	46.00	56.29	56.29	0.00	36.60	63.40	
Kharu musai	6.38	0.15	41.60	56.29	56.29	0.00	42.60	57.40	
Daglatnor	5.37	0.34	61.40	23.42	69.67	43.93	10.90	89.10	
Mohammadi	-	-	-	-	-	-	42.00	58.00	
Gisovan	6.59	0.14	54.00	58.46	58.46	0.00	21.90	78.10	3

Table 6. Some physical characteristics of fruits of date cultivars in different regions of Hadji-Abad (Tezerj, Sirmand, and Ahmadi).

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/ seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Zahedi	31.21	19.99	7.32	6.48	0.81	7.99	19.70	7.22	
Nohdanegazi	51.50	20.50	9.88	9.03	0.86	11.48	20.50	7.50	
Kheneizi	31.76	21.53	10.54	9.81	0.70	14.13	19.61	8.93	
Khasui	25.85	18.81	6.92	63.98	0.49	13.03	15.80	7.53	
Lasht	37.26	21.13	11.16	10.58	0.70	15.31	21.79	6.05	
Medjul	37.31	22.02	11.39	10.44	0.93	11.20	20.68	17.28	
Marzban	38.20	20.91	10.28	9.43	0.79	11.98	22.25	6.42	
Shahani	38.74	17.50	8.02	6.81	0.75	9.65	24.64	5.44	
Mordaseng	26.34	19.85	8.78	8.02	0.64	12.40	16.70	6.54	1
Kabkab	36.60	21.52	9.72	11.07	0.66	17.17	20.08	5.98	
Halavi	35.70	17.51	6.78	5.96	0.80	7.38	20.89	6.42	
Deyri	37.18	19.42	8.30	7.14	1.13	6.30	23.44	7.46	
Piyarom	39.70	17.85	9.16	8.08	1.07	7.47	23.66	6.69	
Negar	35.81	17.04	7.29	6.31	0.95	6.61	23.37	6.71	
Turi	33.72	18.75	6.58	5.63	0.93	6.00	20.55	7.35	
Berhi	27.84	20.62	6.94	6.29	0.63	9.67	16.59	6.67	
Karite	31.26	21.20	10.07	9.03	1.07	8.43	20.50	7.20	
Mozafati	36.78	21.35	12.37	11.49	0.86	13.40	22.08	6.39	
Siahbizu	33.23	17.66	6.45	5.56	0.96	6.24	20.40	6.78	
Zard	34.78	21.90	12.44	11.26	1.17	9.62	23.71	7.12	
Asnaf	30.08	19.36	8.35	7.45	0.90	8.27	21.97	7.48	
Lelengani	35.69	20.08	10.77	9.38	0.88	10.79	22.17	6.57	
Shekari	38.08	22.54	14.84	12.39	1.16	10.68	23.61	6.88	
Kharkalut	28.97	16.96	5.55	4.75	0.78	6.08	19.47	6.70	
Almehtari	27.50	18.80	5.70	5.19	0.50	10.57	15.83	6.70	
Anbari	31.63	18.48	8.85	8.00	0.71	11.26	21.07	5.94	
Kharuzard	30.32	16.36	5.72	4.97	0.73	6.80	19.26	6.65	
Zarek	31.17	22.64	10.73	9.72	0.89	10.92	21.47	6.63	
Torshuk	40.73	18.85	9.49	8.32	1.14	7.29	26.67	6.87	
Paselari	36.04	29.48	18.31	17.23	1.04	16.56	21.67	8.01	
Kalut	29.82	18.69	8.22	6.97	1.13	6.16	19.92	7.69	
Ferekan	34.81	15.90	6.04	5.28	0.73	7.57	22.19	5.80	2
Kharakpiarom	43.47	19.43	13.85	12.75	1.10	11.59	26.70	6.56	
Kharu	26.16	14.37	4.68	3.87	0.78	4.96	17.86	6.93	
Kharushahani	40.86	15.77	6.76	5.87	0.85	6.90	23.04	6.25	
Bedunenam1	38.64	22.60	13.42	12.21	1.05	11.62	24.69	6.14	
Melson	33.15	22.85	7.66	6.95	0.67	10.35	20.68	6.68	
Bardivar	31.63	18.48	8.85	8.00	0.71	11.26	21.07	5.94	
Kharakmozafati	37.33	23.05	14.69	13.45	1.14	11.79	23.85	6.81	
Khanziri	30.89	22.00	12.00	11.11	0.85	13.07	21.41	5.98	
Halili	29.38	21.83	8.62	8.08	0.50	16.23	16.70	6.26	
Bardial	35.75	20.56	10.74	9.80	0.90	10.86	23.96	11.48	
Pasmash	30.32	16.36	5.72	4.97	0.73	6.80	19.26	6.65	
Kharumusai	31.17	22.64	10.73	9.72	0.89	10.92	21.47	6.63	
Daglatnor	38.35	18.47	7.00	6.36	0.63	10.05	22.20	6.40	
Mohammadi	36.00	17.02	8.39	7.36	0.98	7.51	23.75	6.62	
Gisovan	28.91	16.10	6.52	5.56	0.96	5.79	19.72	6.72	3

Table 7. Some chemical characteristics of fruits of date cultivars in Parsian region.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Moosali	5.74	0.33	59.20	50.67	54.28	3.42	14.50	85.50	
Khasui	6.21	0.16	50.60	50.67	53.34	2.53	30.65	69.35	
Azad	5.94	0.30	56.80	54.28	54.28	0.00	21.00	79.00	
Estamaran	6.17	0.22	54.00	58.46	63.34	4.63	22.60	77.40	
Zahedi	5.67	0.27	56.00	63.34	63.34	0.00	19.20	80.80	
Marzbanekiuor	6.24	0.27	59.60	63.34	63.34	0.00	16.50	83.50	
Zamardan	6.06	0.33	50.80	58.46	69.09	10.09	28.90	71.10	
Kheneyzi	5.75	0.33	60.40	63.34	63.34	0.00	15.90	84.10	1
Marzbanedaryai	6.52	0.22	50.20	50.89	54.56	3.49	27.15	72.85	
Kabkab	6.64	0.19	48.20	49.08	50.89	1.71	32.10	67.90	
Barimi	6.66	0.14	36.80	38.00	40.00	1.90	44.50	55.50	
Sameran	6.21	0.16	42.00	50.67	50.67	0.00	34.10	65.90	
Mordaseng	6.49	0.15	49.60	52.41	54.28	1.77	29.60	70.40	
Maktum	7.43	0.10	44.80	47.50	50.67	3.01	35.60	64.40	
Khazravi	6.12	0.15	52.80	63.34	63.34	0.00	-	-	
Ghasab	5.83	0.21	56.40	63.34	63.34	0.00	21.10	78.90	
Michusharif	5.68	0.33	59.20	58.46	58.46	0.00	20.10	79.90	
Hamerun	7.00	0.14	44.00	44.70	54.28	9.10	27.70	72.30	
Shahri	6.06	0.22	48.80	58.46	63.34	4.63	29.60	70.40	
Khoshkong	5.95	0.30	60.40	58.46	63.34	4.63	14.30	85.70	
Fiba	6.09	0.33	63.60	63.34	69.09	5.46	9.00	91.00	
Michotonbeki	5.96	0.25	55.20	54.28	58.46	3.97	22.80	77.20	
Narmchahi	6.30	0.38	64.00	58.46	58.46	0.00	10.30	89.70	
Kharuy-e-Fariab-e-Haji Mohammad	5.31	0.49	64.80	69.09	76.00	6.56	6.90	93.10	2
Piyarom	6.00	0.35	60.00	63.34	63.34	0.00	9.10	90.90	
Khosh kharak	6.30	0.27	60.00	63.34	69.09	5.46	8.50	91.50	
Peypa	7.22	0.08	42.40	46.06	47.50	1.36	38.60	61.40	
Gach kha	6.61	0.10	39.20	42.22	44.70	2.35	45.00	55.00	
Mazrui	6.47	0.10	41.20	44.70	47.50	2.66	43.30	56.70	
Rajunei	6.86	0.16	42.80	44.70	46.06	1.29	41.10	58.90	
Halo	6.75	0.16	40.80	50.67	52.41	1.65	42.40	57.60	3
Parzman key bu	6.94	0.11	46.80	54.28	58.46	3.97	31.40	68.60	

Table 8. Some physical characteristics of fruits of date cultivars in Parsian region.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp Weight (g)	Seed weight (g)	Pulp/ seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Moosali	28.80	17.00	6.03	5.46	0.54	10.12	15.75	6.10	
Khasui	24.90	17.55	5.65	5.00	0.62	8.63	16.05	6.93	
Azad	36.50	18.80	10.11	9.44	0.63	14.98	20.00	5.40	
Estamaran	37.15	16.80	6.68	5.83	0.84	6.94	23.20	6.00	
Zahedi	30.60	17.55	5.03	4.39	0.62	7.08	17.30	6.60	
Marzbanekivor	35.80	17.85	7.08	6.27	0.81	7.74	18.75	6.75	
Zamardan	43.60	18.35	13.12	12.06	1.03	11.70	23.65	6.65	
Kheneyzi	32.10	19.85	7.32	6.67	0.63	10.58	19.85	5.70	
Marzbanedaryai	34.47	17.95	7.24	6.46	0.71	9.01	21.36	6.97	1
Kabkab	38.89	21.49	13.55	12.58	0.95	13.21	22.80	6.39	
Barimi	25.94	17.36	5.70	4.93	0.76	6.48	17.92	6.93	
Sameran	30.09	17.18	5.69	5.02	0.65	7.72	20.47	5.97	
Mordaseng	26.76	18.35	5.90	5.32	0.68	7.82	15.41	6.25	
Maktum	30.55	22.84	11.40	10.46	0.91	11.49	21.08	7.19	
Khazravi	38.69	22.20	15.30	14.23	1.05	13.55	23.46	6.43	
Ghasab	28.90	18.61	6.70	5.83	0.85	6.85	19.39	6.94	
Michusharif	38.25	19.55	9.42	8.63	0.80	10.78	22.15	6.00	
Hamerun	31.55	20.55	8.30	7.50	0.75	10.00	18.10	6.15	
Shahri	40.50	17.85	7.70	6.89	0.79	8.72	20.75	6.40	
Khoshkong	34.15	17.40	7.85	6.99	0.85	8.22	18.75	6.75	
Fiba	30.40	17.90	5.43	4.78	0.66	7.24	18.65	5.55	
Michotonbeki	34.10	17.35	7.51	6.18	1.26	4.90	24.60	7.25	
Narmchahi	28.50	14.10	4.36	3.77	0.58	6.50	17.85	6.25	
Kharuy-e-Fariab-e-Haji Mohammad	32.20	19.40	8.18	7.30	0.91	8.02	18.75	8.00	2
Piyarom	32.40	18.35	8.65	8.00	0.59	13.55	18.75	5.80	
Khosh kharak	34.15	15.05	6.49	5.51	1.03	5.35	21.80	6.80	
Peypa	32.49	23.50	12.21	11.25	0.95	11.84	22.77	6.98	
Gach kha	32.60	21.19	10.04	9.23	0.79	11.68	18.53	6.92	
Mazrui	31.02	20.67	10.35	9.54	0.79	12.07	21.18	6.29	
Rajunei	36.70	19.24	10.40	9.07	1.21	7.49	21.99	7.43	
Halo	32.94	24.16	12.16	11.08	1.04	10.65	22.20	7.31	3
Parzman key bu	36.53	20.68	10.70	9.75	0.79	12.34	22.41	6.02	

Table 9. Some chemical characteristics of fruits of date cultivars in Bandare-e-Lengeh region.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Khuneyzi	5.84	0.31	54.66	55.86	58.69	2.68	22.20	77.70	
Zamardan	6.50	0.19	52.00	59.81	59.81	0.00	28.45	71.55	
Gentar	6.32	0.30	55.20	58.46	58.46	0.00	24.60	75.40	
Berhi	6.41	0.27	55.60	63.34	63.34	0.00	26.10	73.90	
Mordaseng	6.68	0.25	51.40	59.63	62.07	2.31	24.60	75.40	1
Moseli	6.02	0.25	48.20	55.42	55.42	0.00	30.45	69.55	
Marzban	6.72	0.14	50.80	54.28	54.28	0.00	25.50	74.50	
Azad	7.02	0.14	46.00	47.50	50.67	3.01	35.10	64.90	
Shaham	6.22	0.11	50.00	54.28	56.29	1.90	25.40	74.60	
Gach kha	6.82	0.14	53.20	63.34	63.34	0.00	25.30	74.70	
Kharuposhtebagh	6.62	0.23	50.80	47.32	51.67	4.12	31.10	68.85	
Khosravi	5.61	0.27	43.60	44.70	44.70	0.00	36.00	64.00	
Sorkhdang	6.49	0.22	50.80	57.00	57.00	0.00	24.95	75.05	
Pibayejamil	6.28	0.33	53.20	58.46	63.34	4.63	25.00	75.00	
Shahani	6.00	0.30	52.80	58.46	63.34	4.63	25.10	74.90	2
Kabkab	6.82	0.14	47.20	47.50	47.50	0.00	29.00	71.00	
Shekarpare	6.74	0.14	49.60	54.28	54.28	0.00	27.50	72.50	
Bolduz	6.30	0.22	54.80	56.29	58.46	2.06	19.90	80.10	

Table 10. Some physical characteristics of fruits of date cultivars in Bandare-e-Lengeh region.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/ seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Khuneyzi	29.41	19.08	8.75	7.74	0.94	8.53	17.78	6.88	
Zamardan	33.47	16.55	7.08	6.36	0.71	9.01	18.59	6.91	
Gentar	28.65	16.50	5.91	5.08	0.83	6.12	18.00	6.50	
Berhi	24.45	17.20	6.61	6.00	0.63	9.52	14.80	6.70	
Mordaseng	23.86	17.36	6.00	5.25	0.73	7.15	16.55	7.48	
Moseli	33.83	18.25	9.94	8.90	1.09	8.17	19.55	8.08	1
Marzban	29.50	16.70	6.58	5.96	0.59	10.10	17.32	7.35	
Azad	33.52	19.85	9.63	8.92	0.66	13.51	19.98	7.37	
Shaham	33.52	17.24	8.10	7.35	0.71	10.35	18.35	7.65	
Gach khab	32.50	19.50	8.61	7.62	0.98	7.77	19.82	8.60	
KharuPoshteBagh	29.38	17.35	7.53	6.61	0.86	7.66	19.02	7.75	
Khosravi	27.40	21.50	10.46	9.15	1.31	6.98	21.10	7.30	
Sorkhdang	31.50	19.70	8.34	7.68	0.63	12.57	19.35	7.16	
Pibayejamil	31.50	14.45	5.23	4.42	0.85	5.20	19.30	6.40	
Shahani	40.30	15.20	7.19	6.28	0.89	7.05	26.00	5.80	2
Kabkab	37.35	23.85	15.78	14.52	1.21	12.00	24.35	6.85	
Shekarpare	33.62	17.70	7.77	7.17	0.59	12.15	20.40	7.15	
Bolduz	34.84	19.57	9.21	8.09	1.00	8.09	19.42	8.57	

Table 11. Some chemical characteristics of fruits of date cultivars in Bastak region.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Lasht	6.70	0.16	52.80	44.70	47.50	2.66	12.70	87.30	1
Berhi	5.95	0.30	60.20	44.41	46.24	1.73	17.80	82.20	
Marzban	5.49	0.38	56.00	47.50	58.46	10.41	14.90	85.10	
Azar	6.40	0.22	56.80	63.34	63.34	0.00	13.20	86.80	
Mozafati	6.66	0.22	52.80	58.46	63.34	4.63	14.10	85.90	
Khalas	6.13	0.30	64.00	58.46	58.46	0.00	13.60	86.40	
Mosali	5.84	0.27	53.00	51.58	52.98	1.33	40.90	59.10	
Khasui	6.80	0.20	53.40	51.89	56.37	4.25	15.50	84.50	
Mordaseng	6.79	0.18	52.00	57.87	61.68	3.61	23.00	77.00	
Zardelar	5.79	0.41	62.80	50.67	54.28	3.42	9.00	91.00	
Kheneyzi	7.06	0.16	50.00	56.29	56.29	0.00	33.20	66.80	
Barim	6.15	0.22	49.60	54.28	54.28	0.00	33.80	66.20	
Estamaran	6.86	0.14	49.60	52.41	54.28	1.77	34.30	65.70	
Shahani	6.24	0.30	56.80	60.90	66.21	5.04	21.20	78.80	
Shishi	6.29	0.38	66.40	63.34	63.34	0.00	14.10	85.90	2
Gamsari	6.10	0.37	59.00	56.37	63.77	7.03	24.35	75.65	
Lolo	5.86	0.33	62.80	63.34	76.00	12.02	14.50	85.50	
Begshi	6.05	0.30	64.00	63.34	69.09	5.46	10.20	89.80	
Gachkha	6.68	0.18	55.40	55.42	57.00	1.50	26.20	73.80	
Kharuyesedreza	6.21	0.19	64.00	58.46	63.34	4.63	13.50	86.50	
Fiba	6.09	0.33	36.60	63.34	69.09	5.46	9.00	91.00	
Darvishsahmadi	5.91	0.43	62.00	54.28	54.28	0.00	11.10	88.90	
Gentar	5.81	0.46	64.00	58.46	63.34	4.63	6.10	93.90	
Narmchahi	6.3	0.38	64.80	58.46	58.46	0.00	10.30	89.70	
Kharuksedrezai	6.79	0.19	49.60	56.29	58.46	2.06	27.10	72.90	
Peyva	6.46	0.27	52.80	56.29	58.46	2.06	27.00	73.00	
Khoshkharak	7.15	0.14	48.00	50.67	50.67	0.00	41.40	58.60	
Piarom	7.00	0.14	52.80	58.46	58.46	0.00	29.70	70.30	
Halili	5.87	0.41	63.20	58.46	63.34	4.63	11.70	88.30	3
Kharuy-e-Fariab-e-Haji Mohammad	5.31	0.49	64.80	69.09	76.00	6.56	6.90	93.10	
Halo	6.79	0.16	50.00	56.29	58.46	2.06	32.30	67.70	
Kharuk Posht-e-Bagh	6.90	0.23	53.60	56.29	58.46	2.06	35.70	64.30	

Table 12. Some physical characteristics of fruits of date cultivars in Bastak region.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/ seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Lasht	35.50	19.30	7.09	6.39	0.69	9.26	20.90	5.95	
Berhi	28.47	20.10	7.16	6.64	0.78	8.68	17.63	6.96	
Marzban	39.20	20.75	10.44	9.54	0.92	10.36	21.10	6.85	
Azar	30.10	17.55	7.29	6.75	0.57	11.84	18.00	5.75	
Mozafati	39.75	18.45	10.62	9.78	0.78	12.53	21.40	6.00	
Khalas	34.00	16.75	8.06	7.31	0.75	9.74	20.60	6.20	
Mosali	32.54	19.47	9.07	8.27	0.79	10.91	18.60	6.66	1
Khasui	25.06	18.20	6.26	5.78	0.48	11.89	15.00	5.67	
Mordaseng	21.73	16.61	5.65	5.02	0.59	8.43	14.97	6.91	
Zardelar	28.55	17.30	5.13	4.51	0.63	7.15	17.65	6.55	
Kheneyzi	25.75	18.27	6.43	5.82	0.60	9.70	16.30	6.66	
Barim	26.55	18.89	7.18	6.29	0.88	7.14	16.27	6.75	
Estamaran	36.22	17.05	8.06	6.98	1.01	6.91	22.77	6.69	
Shahani	35.49	14.38	5.41	4.72	0.65	7.38	21.99	5.57	
Shishi	35.40	15.00	8.43	7.70	0.73	10.54	20.00	6.35	
Gamsari	28.16	17.98	7.50	6.76	0.73	9.22	18.30	6.68	
Lolo	22.75	18.50	5.60	4.82	0.77	6.25	15.75	7.65	
Begshi	34.15	12.20	4.43	3.97	0.46	8.63	17.35	5.25	
Gachkha	29.12	17.46	7.64	6.93	0.70	9.86	17.37	6.63	
Kharuyesedreza	37.60	17.85	9.30	8.15	1.13	7.21	21.15	7.50	
fiba	30.40	17.90	5.43	4.78	0.66	7.24	18.65	5.55	2
Darvishsahmadi	35.60	21.20	11.33	10.40	0.93	11.18	19.70	7.50	
Gentar	22.35	17.85	4.89	4.35	0.54	8.05	14.65	6.85	
Narmchahi	28.50	14.10	4.36	3.77	0.48	6.50	17.85	6.25	
Kharuksedrezai	34.92	18.89	9.68	8.61	1.07	8.04	20.48	7.46	
Peyva	32.70	19.07	9.48	8.83	0.65	13.58	17.75	5.57	
Khoshkharak	32.07	22.20	9.50	8.55	0.93	9.19	20.27	7.14	
Piarom	35.32	16.77	7.59	6.57	1.02	6.44	22.08	6.53	
Halili	35.75	17.65	10.15	9.28	0.90	10.31	22.40	6.70	
Kharuy-e-Fariab-e-Haji Mohammad	32.20	19.40	8.18	7.30	0.91	8.02	18.75	8.00	3
Halo	30.66	18.56	7.76	7.06	0.70	10.08	19.23	6.58	
Kharuk Posht-e-Bagh	29.55	17.33	5.83	5.09	0.74	6.86	18.19	6.53	

Table 13. Some chemical characteristics of fruits of date cultivars in Fin-o-Rezvan region.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Lashti	6.16	0.24	50.40	43.46	47.50	3.83	32.25	67.75	
Negar	5.94	0.23	51.10	47.27	54.40	8.16	24.37	75.62	
Khasui	6.89	0.19	53.20	57.30	57.30	0.00	24.43	75.56	
Khasab	6.86	0.12	48.40	54.28	54.28	0.00	31.40	68.60	
Mangenas	6.36	0.30	55.00	57.00	65.14	7.72	15.45	84.05	
Almehtari	6.42	0.30	59.00	50.89	60.90	9.50	16.75	83.25	
Mozafati	6.76	0.20	53.33	49.61	50.67	1.00	30.63	69.03	
Nabati	5.97	0.33	60.00	63.34	63.34	0.00	17.80	82.20	
Turi	5.49	0.27	49.60	58.46	58.46	0.00	17.30	82.70	1
Zahedi	5.92	0.25	62.40	60.00	67.05	6.69	20.50	79.50	
Kariteh	7.11	0.14	48.00	47.50	49.03	1.45	34.20	65.80	
Kheneyzi	6.37	0.23	52.80	51.93	52.78	0.80	22.33	77.66	
Marzban	6.60	0.21	51.80	50.89	52.98	1.98	20.40	79.60	
Hasan Mehdi	6.18	0.22	52.80	50.67	50.67	0.00	30.30	69.70	
Museli	6.20	0.19	49.00	49.77	51.58	1.71	25.00	75.00	
Mordaseng	6.15	0.33	58.00	58.46	63.34	4.63	12.50	87.50	
Zohrei	6.66	0.31	51.80	49.49	51.58	1.98	21.75	78.25	
Paruzkhesh	7.40	0.16	44.00	50.67	50.67	0.00	32.50	67.50	
Hasht Danegazi	6.67	0.19	43.20	40.00	42.23	2.11	41.90	58.10	
Narshotori	5.95	0.35	60.00	58.46	58.46	0.00	17.50	82.50	
Gavansuri	5.78	0.38	60.00	69.09	69.09	0.00	13.10	86.90	
Shahani	6.28	0.21	52.40	50.67	54.56	3.70	24.85	66.15	
Mono	6.36	0.30	55.86	58.69	62.23	3.36	20.73	79.26	
Surdang	6.51	0.23	51.86	47.02	54.86	7.43	24.56	75.43	
Kalaksorkh	6.19	0.27	63.20	63.34	69.09	5.46	9.20	90.80	
Khorestnegar	6.00	0.41	62.00	76.00	76.00	0.00	24.00	76.00	
Khorest Shahani	5.90	0.43	62.00	76.00	76.00	0.00	12.30	87.70	
Sarpola	6.05	0.33	56.00	63.34	69.00	5.37	20.70	79.30	2
Daglatnor	6.17	0.22	61.20	60.00	63.34	3.17	22.30	77.70	
Shekari	6.38	0.30	56.00	54.28	54.28	0.00	20.90	79.10	
Shahri	6.03	0.27	58.00	58.46	58.46	0.00	18.80	81.20	
Paselari	6.52	0.16	45.60	52.41	52.41	0.00	36.00	64.00	
Piyarom	5.35	0.39	60.80	66.17	72.54	6.05	13.20	86.80	
Ghalami	6.50	0.26	54.60	61.68	63.77	1.98	23.10	76.90	
Maranguni	6.44	0.16	48.40	50.67	52.47	1.71	32.00	68.00	
Beskahti	5.92	0.31	59.80	60.90	65.42	4.29	15.45	84.55	
Khorest Marzban	6.18	0.30	56.80	54.28	54.28	0.00	12.60	87.40	
Seloni	6.57	0.19	57.60	50.67	54.28	3.42	20.70	79.30	
Zard	6.30	0.23	50.20	49.08	54.56	5.20	30.70	69.30	
Toranji	6.43	0.29	54.00	49.49	52.47	2.83	16.35	83.65	
Beskahti2	6.93	0.10	49.60	47.50	50.67	3.01	30.00	70.00	
Torshaki	7.26	0.14	49.60	50.67	50.67	0.00	30.20	69.80	
Angoshtarus	5.99	0.30	64.00	63.34	63.34	0.00	8.40	91.60	
Beskahti1	7.27	0.16	44.00	50.67	44.70	5.67	32.20	67.80	
Poshtbani	6.12	0.27	58.00	44.70	58.46	13.07	11.70	88.30	3
Shakhti	6.24	0.42	56.00	49.49	56.37	6.53	16.15	83.85	
Zarek	6.56	0.24	54.53	57.06	62.23	4.90	21.70	78.30	
Aghai	6.63	0.29	56.40	50.89	50.89	0.00	26.85	73.15	
Kongerd	6.60	0.29	55.06	61.03	65.45	4.19	21.03	78.96	
Bi Nam	5.78	0.67	62.40	63.34	76.00	12.02	-	-	
Shenasai Nashode	6.29	0.19	50.40	50.67	54.28	3.42	14.40	85.60	

Table 14. Some physical characteristics of fruits of date cultivars in Fin-o-Rezvan region.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/ seed ratio	Seed length (mm)	Seed diameter (mm)	Quality
Lashti	43.16	19.04	13.53	12.44	1.08	11.41	27.70	7.35	
Negar	37.24	18.67	9.50	8.42	1.00	8.84	22.50	8.13	
Khasui	27.18	16.34	6.30	5.65	0.63	9.95	18.01	6.44	
Khasab	25.94	19.34	8.88	8.21	0.61	13.45	17.52	6.62	
Mangenas	30.25	14.87	5.54	4.62	0.91	5.08	21.94	6.56	
Almehtari	27.21	14.44	4.29	3.48	0.8	4.40	20.31	7.18	
Mozafati	32.28	19.53	10.11	9.11	0.93	9.84	20.12	7.25	
Nabati	27.09	16.55	6.05	5.27	0.76	6.90	17.54	7.24	
Turi	26.98	16.68	4.72	3.58	0.80	4.47	16.36	7.23	1
Zahedi	32.22	20.42	8.73	7.97	0.76	10.48	17.82	7.16	
Kariteh	33.00	21.01	11.35	10.4	0.94	11.06	20.92	6.94	
Kheneyzi	29.96	18.75	8.08	7.48	0.58	12.64	19.87	6.27	
Marzban	32.19	17.89	7.83	7.03	0.78	9.00	20.35	7.29	
Hasan Mehdi	31.10	17.62	7.97	7.11	0.85	8.36	20.46	7.92	
Museli	29.13	13.38	12.64	11.72	0.90	13.08	17.32	6.61	
Mordaseng	25.00	18.10	7.00	6.40	0.70	9.14	17.60	6.30	
Zohrei	27.84	11.75	8.60	7.66	0.92	8.91	20.32	7.48	
Paruzkhesh	36.28	17.03	8.18	7.40	0.77	9.61	23.36	7.63	
Hasht Danegazi	40.06	19.42	12.92	11.58	1.34	8.64	23.14	6.19	
Narshotori	29.62	13.89	4.45	3.72	0.71	5.24	19.90	6.47	
Gavansuri	29.71	17.04	7.21	6.36	0.85	7.50	19.35	5.69	
Shahani	37.73	14.63	6.55	5.77	0.78	4.11	24.85	5.94	
Mono	36.68	19.73	10.29	9.67	0.78	12.45	18.86	7.10	
Surdang	32.39	20.33	9.58	8.85	0.72	12.11	19.99	6.30	
Kalak Sorkh	27.90	13.57	4.51	3.56	0.94	3.79	21.20	6.66	
Khorestnegar	38.00	16.30	9.10	8.01	1.05	7.62	22.10	7.11	
Khorest Shahani	35.71	13.58	5.55	4.83	0.74	6.52	17.15	5.87	2
Sarpola	28.94	17.34	7.30	6.55	0.69	9.47	18.81	6.72	
Daglatnor	36.77	16.80	6.81	5.76	1.24	4.64	25.06	7.37	
Shekari	28.70	16.20	6.036	5.22	0.79	6.64	20.70	6.50	
Shahri	35.17	16.70	6.81	5.93	0.86	6.89	21.85	7.77	
Paselari	25.38	21.91	9.09	8.35	0.72	11.59	18.83	6.80	
Piarom	35.71	15.26	6.51	5.52	0.99	5.60	21.87	6.22	
Ghalami	26.26	21.52	10.28	8.57	0.80	10.68	17.20	6.51	
Maranguni	34.05	17.47	8.00	7.23	0.73	9.90	20.97	6.73	
Beskahti	29.40	17.17	5.80	5.04	0.75	6.70	18.56	6.60	
Khorest Marzban	33.16	16.63	6.05	5.27	0.85	6.20	18.01	6.87	
Seloni	25.55	17.04	5.64	4.95	0.68	7.27	17.58	7.27	
Zard	30.21	17.47	7.30	6.70	0.60	11.09	17.73	6.81	
Toranji	24.45	16.38	5.20	4.44	0.77	6.08	17.94	7.57	
Beskahti2	27.28	17.00	7.30	6.39	0.83	7.69	18.38	8.23	
Torshaki	25.92	18.70	7.36	6.66	0.69	9.65	16.65	8.15	
Angoshtarus	36.3	15.98	6.59	6.46	0.70	9.27	24.17	5.83	
Beskahti1	31.16	20.25	10.98	10.03	0.95	10.55	24.05	7.18	
Poshtbani	31.37	18.09	7.11	6.15	0.96	6.37	21.60	7.66	3
Shakhti	36.50	15.62	7.02	6.15	0.87	7.07	23.59	7.21	
Zarek	32.72	15.52	5.87	5.08	0.75	6.72	22.27	6.04	
Aghai	30.02	17.63	6.04	5.34	0.67	7.89	19.05	6.56	
Kongerd	31.56	18.46	7.62	6.77	0.84	8.43	21.02	6.97	
Bi Nam	29.40	17.17	5.80	5.04	0.75	6.70	18.56	6.6	
Shenasai Nashode	23.67	17.33	6.35	5.72	0.60	9.48	16.35	6.48	

Table 15. Some chemical characteristics of fruits of date cultivars Siyahoo region.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Farz	6.10	026	48.00	50.67	54.28	3.42	33.00	67.00	
Mozafati	5.89	024	44.00	47.50	47.50	0.00	35.40	64.60	
Marzeban	5.59	0.37	57.60	46.58	47.50	0.87	19.00	81.00	1
Kariteh	6.82	0.21	51.20	54.28	63.34	8.60	29.30	70.70	
Khasooei	6.90	0.21	54.00	42.23	58.46	15.41	24.60	75.40	
Khenuzi	6.34	0.28	57.00	60.90	60.9	0.00	21.10	78.90	
Khosh Kong	6.91	0.26	48.80	50.67	58.46	7.40	24.90	75.10	
Bardivar	6.06	0.29	54.80	63.34	69.09	5.46	13.40	86.60	
Shahani	6.08	0.34	60.80	63.77	69.09	5.04	11.75	88.24	
Kharkaloot	6.06	0.32	52.40	54.28	69.09	14.06	17.00	83.00	
Khodroo sefid	6.00	0.45	52.00	58.46	58.46	0.00	14.63	85.37	2
Kong Gerd	6.19	0.32	60.00	58.46	58.46	0.00	11.71	88.28	
Baskahti	6.54	0.24	61.20	58.46	63.34	4.63	17.22	82.77	
Merengani	6.23	0.26	56.00	58.46	58.46	0.00	15.76	84.23	
Khoroosiyah	6.34	0.24	58.80	50.67	63.34	12.03	16.54	83.45	
Zarkamaneh	6.22	0.29	59.20	58.46	76.00	16.66	11.56	88.43	

Table 16. Some physical characteristics of fruits of date cultivars Siyahoo region.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/seed ratio	Seed length (mm)	Seed diameter (mm	Quality
Farz	35.53	20.00	11.00	10.30	0.50	20.6	18.40	4.50	
Mozafati	26.10	17.30	12.97	12.10	0.80	15.22	21.20	6.00	
Marzeban	32.03	17.10	7.10	6.20	0.70	8.85	21.00	6.20	1
Kariteh	32.00	16.98	8.10	7.30	0.69	10.73	19.80	5.40	
Khasooei	27.00	13.00	4.00	3.00	0.93	3.22	20.10	6.00	
Khenuzi	30.94	17.32	8.65	8.02	0.57	14.18	19.14	5.89	
Khosh Konge	31.00	16.10	6.97	6.00	0.90	6.74	22.00	6.96	
Bardivar	35.00	17.40	8.10	7.80	0.80	9.75	23.11	5.10	
Shahani	39.97	13.80	7.24	6.48	0.74	8.72	21.16	5.59	
Kharkaloot	-	-	-	-	-	-	-	-	
Khodroo sefid	38.42	16.65	5.88	5.15	0.73	7.05	24.02	6.07	2
Kong Gerd	33.75	16.20	3.75	6.77	0.97	6.89	23.23	6.09	
Baskahti	30.37	16.85	7.03	6.19	0.83	7.38	19.03	6.17	
Merengani	30.77	20.98	8.17	7.32	0.72	10.16	18.94	7.07	
Khoroosiyah	34.43	18.73	8.79	7.825	0.89	9.79	19.39	6.42	
Zarkamaneh	34.79	14.86	6.29	5.60	0.64	8.75	19.70	5.90	

Table 17. Chemical characteristics of fruits of date cultivars in Qeshem island.

Cultivar	pH	Acidity (%)	TSS (%)	Reducing sugars (%)	Total sugar (%)	sucrose (%)	Moisture content (%)	Dry matter (%)	Quality
Khasab	7.13	0.18	46.00	44.70	44.70	0.00	29.30	70.70	
Mordaseng	6.97	0.16	45.60	44.70	44.70	0.00	35.30	64.70	1
Berhee	7.18	0.26	49.60	47.50	47.50	0.00	34.30	65.70	
Marzeban	7.25	0.14	49.20	44.70	46.06	1.29	29.90	70.10	
Karoo Azari	7.07	0.14	49.60	49.03	50.67	1.55	28.50	71.50	2
loloei	7.23	0.18	44.80	42.23	44.70	2.34	39.20	60.80	
Kharook Giri	6.68	0.18	46.80	46.50	49.03	1.45	33.80	66.20	
Kharook Qeshmi	6.88	0.14	47.60	49.49	50.89	1.33	30.50	69.50	3
Zarek	7.15	0.18	47.20	46.06	49.03	2.82	35.80	64.20	

Table 18. Some physical characteristics of fruits of date cultivars Qeshem island.

Cultivar	Fruit length (mm)	Fruit diameter (mm)	Fw of single fruit (g)	Pulp weight (g)	Seed weight (g)	Pulp/ seed ratio	seed length (mm)	seed diameter (mm)	Quality
Khasab	25.16	16.13	5.37	4.89	0.47	10.40	15.23	5.94	
Mordaseng	19.40	14.80	4.18	3.63	0.60	6.05	14.91	6.26	1
Berhee	19.50	13.77	3.22	2.32	0.90	2.58	13.50	6.34	
Marzeban	32.55	20.86	7.67	6.76	1.00	6.76	19.60	6.51	
Karoo Azari	32.17	15.90	6.37	5.47	0.89	6.14	21.41	6.53	2
loloei	25.73	18.57	6.83	5.86	0.97	6.04	17.40	7.97	
Kharook Giri	30.65	18.17	8.37	7.47	0.90	8.30	18.07	6.75	3
Kharook Qeshmi	31.46	18.66	8.56	7.67	0.88	8.71	19.41	7.07	
Zarek	25.94	13.85	4.06	3.28	0.77	4.25	19.43	7.06	

Figures

Fig. 1. New genotypes of the Iranian date germplasm. (a) ‘Nohdaneh Gazi’, (b) ‘Hassan Mahdi’, (c) ‘Moghbali Modaldal’.

Efficiency and Longevity of Food Baits in Palm Weevil Traps

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Keywords: *Rhynchophorus ferrugineus*, *Rhynchophorus palmarum*, pheromone, kairomone, trapping

Abstract

Pheromone trapping of RPW is an effective method to manage populations of this palm pest. Current pheromone lures last for 2 to 5 months depending on the season, but the necessary food component lasts only for a week or two. Water evaporation and loss of attractancy are the two main problems with the food component. This paper will present work done on a related species *Rhynchophorus palmarm* aimed at making the food component of the trap more attractive as well as adding ingredients to the trap to extend the field life of the food. Emission of ethyl acetate from dispensers in pheromone/food traps increases captures compared to pheromone/food traps by 2-5×. Addition of propylene glycol to traps extends the effective life of food in traps. What has not been effective in the field to date is the substitution of attractive blends that attempt to replace food.

INTRODUCTION

Rhynchophorus palmarum is managed in Central and South America without insecticide spray by pheromone trapping and sanitation practices in oil, coconut and palmito palm. Since it is a strong flyer traps are normally placed at densities of 1 trap per 3 to 7 hectare (Chinchilla et al., 1993). This density of traps removes >80% of weevils during one year (Chinchilla et al., 1993). Traps are plastic containers tied to palms at chest height and are baited with the male-produced aggregation pheromone and insecticide treated sugarcane or palm pieces (Oehlschlager et al., 1993a). Decomposition and desiccation of food bait decreases attraction to traps so food bait is replaced every 2-3 weeks in most trapping programs. Pheromone and kairomone lures are replaced at 3-4 month intervals.

This paper summarizes studies undertaken to decrease decomposition and desiccation of food bait in *R. palmarum* traps. In the early 1990's an initial trial demonstrated that addition of ethyl acetate to pheromone/sugarcane baited traps increased capture of *R. palmarum* (Jaffe et al., 1993). We repeated this work in extensive trials in the mid 1990's and confirmed that emission of ethyl acetate from pheromone/sugarcane baited traps increased captures of *R. palmarum* by 50-100% (Chinchilla and Oehlschlager, unpublished). This experiment was repeated for *R. ferrugineus* in the UAE in 1997 with spectacular success. In the UAE emission of ethyl acetate from pheromone/food baited traps increased capture of *R. ferrugineus* by 2.6×. In 1998 an Egyptian test revealed that emission of ethyl acetate increased capture of *R. ferrugineus* in pheromone/sugarcane baited traps by 5× (Oehlschlager, 1998).

MATERIALS AND METHODS

The initial experiments sought to determine the effect of emission of ethyl acetate from pheromone/sugarcane traps (Jaffe et al., 1993). In this test (Fig. 1) we examined addition of ethyl acetate or ethyl acetate:ethanol (1:1) to pheromone/sugarcane traps. A complete random block design was used. In Costa Rica traps were 20-L white plastic buckets with 4 openings (5×8 cm) near the top. Traps were hung on coconut palms in a 50 ha mature coconut plantation in which about 20% of all trees were removed prior to

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the experiment. Traps were 1.5 m above ground at 50 m intervals with no trap closer than 25 m from any border. All traps contained pheromone lures emitting ~3 mg of 6-methylhept-2-en-4-ol, the aggregation pheromone of *R. palmarum* (Oehlschlager et al., 1992). All traps also contained 5 halved pieces of 20 cm long sugarcane pre-immersed in a 1% lannate (S-methyl-N-[(methylcarbamoyl) oxy] thioacetimidate) solution for 5 min prior to addition of traps. Ethyl acetate was released from plastic bottles with restrictive orifices at 200-400 mg/day. Ethyl acetate-ethanol (1:1) was released from capillary devices at 200-400 mg/day such that the two components were released at a constant ratio. Insects were collected and removed weekly at which time treatment positions were re-randomized. The UAE experiment (Fig. 2) was conducted in a mature date palm plantation of about 3 ha using 12-L traps with molded rough surface on the exterior (Emirates Overseas Group, Abu Dhabi) and 4 5×8 cm square openings in the sides near the top of each trap. Traps were buried in the ground to the level of the entry holes. Traps were baited with Ferrolure + 700 mg lures which emitted 3-10 mg of 4-methyl-5-nonanol and 4-methyl-5-nonanone (9:1), about 50 g of mashed date fruit in 2-3 L of water and an ethyl acetate dispenser that consisted of a membrane through which the ethyl acetate evaporated at 200-400 mg/day. The experiment in Egypt was conducted in a mature date palm plantation using 12-L bucket traps that had the outer surface covered with palm mat and were placed on the ground. Bucket traps had 4 entry holes of approximately 5×9 cm in the sides near the top. Traps were baited with the same pheromone and ethyl acetate dispensers used in the UAE but contained about 50 g of bigas covered with molasses and about 1 L of water containing 1-3% lannate.

The insecticide vs. no insecticide experiment (Fig. 4) was conducted in Costa Rica in the same 50 ha coconut plantation as used above and employed the same traps, positioning and spacing of traps, pheromone dispensers, food bait and insecticide as described for the experiment in Figure 1.

Experiments in Figures 5, 6 and 7 were conducted in the same 50 ha of commercial coconut palm in Costa Rica. Traps were 12-L white plastic buckets with four 5×10 cm slots in the sides near the top. Pheromone and kairomone lures were hung from the bottom side of lids. Traps were strapped to the palms at 1.5 m above ground 100 m apart and 50 m from any border. Traps contained commercial pheromone lures (ChemTica, Rhyncolure, 750 mg, release rate 3-5 mg/day) and where sugarcane is indicated contained 5 pieces of 20 cm long halved sugarcane. Ethanol was released from membrane lures at 50 mg/day and ethyl acetate (Weevil Magnet) was released from membrane lures at 200-400 mg/day) unless otherwise specified. Traps were left in place for a minimum of 1 week at which time insects were counted and removed. In the case of trap longevity studies no water or other additives were replaced during the course of an experiment. Analysis of capture data was by SYSTAT 9. Means were tested for significant differences by the Bonferonni t-test, $P>0.95$.

The experiment (Fig. 5) that examined blend H as reported by Rochat et al. (2000) as a replacement for sugarcane in pheromone-sugarcane traps was conducted as follows. Blend H is reported to be composed of 28 volatile components in ethyl acetate:ethanol (1:1). The blend was constructed according to Rochat et al. (2000) of (volume %) ethyl acetate (400), ethanol (400, absolute), acetaldehyde (5), methanol (1), acetic acid (7, 90% aqueous solution), isopentanol (5), 2-methylbutanol (1), ethyl propionate (5), propyl acetate (5), isobutyl acetate (13), ethyl butyrate (5), isobutyl propionate (1), ethyl isovalerate (1), isoamyl acetate (5), acetoin (44, dimer), 2,3-butanediol (18, mixture of stereoisomers), 2-hexanone (9), 2-heptanone (2), ethyl 3,3-dimethylacrylate (2), ethyl tiglate (2), phenol (9), alpha-phellandrene (2, >90%), guaiacol (9), 2-nonanone (4), 2-phenylethanol (13), menthone (1, >90%), ethyl octanoate (1) and ethyl decanoate (1). Ethyl acetate, ethanol, methanol and acetic acid were purchased locally. Ethyl octanoate and ethyl decanoate were prepared from ethanol and the corresponding alkyl acid. All other chemicals were purchased and used as obtained from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). The composition of blend H was verified by gas chromatography/mass spectroscopy using a Hewlett Packard 6890 gas chromatograph

linked to a MS 5973 mass selective detector. Chromatographic separation was on a DB-5 column (30 m × 0.25 mm; 0.25 micrometer; J & W Scientific, Folsom, California, USA) that was temperature programmed at 70°C for 4 min, increased to 230°C at 10°C/min and finally held at this temperature for 10 min. Helium was used as the carrier gas at 0.7 ml/min. Analysis of the blend prior to and after field use revealed the relative concentrations of components unchanged within an experimental error of 2% relative to initial values. Release of blend H was via a capillary device calibrated to release 1,500 mg/day as reported by Rochat et al. (2000). The device allowed evaporation of all components in the ratio in which they were in the original blend. Ethyl acetate and ethyl acetate:ethanol lures released 1,500 mg/day. Pheromone lures were as above. Control traps contained a pheromone lure and 10 pieces of halved 20 cm long sugarcane stalk on a 3 cm bed of polyurethane chips (Rochat et al., 2000) to which was added 500 ml 1% lannate solution. Other treatments contained a pheromone lure, a 6 cm bed of polyurethane chips saturated with 1 L of 1% lannate solution (chips were wetted thoroughly with lannate solution) and a lure containing either ethyl acetate:ethanol, ethyl acetate or blend H. Lures were hung from inside the lid of traps. Insects were collected at day 3, 6, 9, 12, 16, and 20 of the trial. At each collection 500 ml of water was added to each trap.

RESULTS AND DISCUSSION

The initial experiment (Fig. 1) examined the ability of ethyl acetate to increase captures of *R. palmarum* to pheromone/sugarcane baited traps. This was based on a report that the emission of an unspecified amount of ethyl acetate from pheromone/sugarcane traps increased capture rates (Jaffe et al., 1993). The use of ethyl acetate in pheromone/sugarcane traps has not been adopted in trapping *R. palmarum* in Central and South America since these reports and we sought to clarify the benefit derived from the addition of this additional attractant to the standard pheromone/sugarcane traps.

We also included in this experiment an ethyl acetate/ethanol combination. This was based on a report that a combination of these two potential attractants improved captures of *R. palmarum* to pheromone/sugarcane traps (Rochat et al., 2000).

Figure 1 shows *Rhynchophorus palmarum* capture in pheromone and sugarcane baited traps additionally emitting ethyl acetate and ethanol. This test was conducted using traps made from 20-L white plastic buckets with four 5×8 cm slots near the top for insect entry. One liter 1% lannate was added to each trap at the start of test and 500 ml after the first week. Treatments were fresh sugarcane and ethyl acetate:ethanol lure; fresh sugarcane and ethyl acetate lure; fresh sugarcane and ethanol lure or fresh sugarcane (control). Weevils were counted and removed after the first week at which time trap positions were rerandomized. The test was conducted for 3 weeks. ANOVA (n=20) revealed no significant differences between treatments.

In this experiment while no significant difference was found between captures in pheromone/sugarcane baited traps and traps baited with pheromone/sugarcane and emitting either ethyl acetate or ethyl acetate and ethanol there was a numerical difference. Experiments with related curculionidae (*Metamasius hemipterus*, Perez et al., 1996) have revealed that ethyl acetate is a potent synergist for pheromone/sugarcane baited traps.

Similar experiments were conducted in Egypt and the United Arab Emirates to examine the effect of emitting ethyl acetate from ground level traps baited with pheromone (Ferrolure^{+}) and food on the capture of *R. ferrugineus*. In the UAE emission of ethyl acetate from pheromone/food traps significantly increased (2.6×) attraction of *R. ferrugineus* (experiment by Aswar and Oehlschlager, Oehlschlager, 1998, Fig. 2). In Egypt emission of ethyl acetate to pheromone/food traps increased captures of *R. ferrugineus* by 5×. (Oehlschlager, 1998, Fig. 2).

Figure 2 shows the capture of *R. ferrugineus* in UAE and Egypt in pheromone and food traps additionally emitting ethyl acetate. The UAE (Aswar and Oehlschlager) experiment was conducted using date bits (~50 g) as food component. The Egyptian experiment (G. Moawad and Y. El Sebay, PPRI, Cairo) was conducted using bigas and

molasses with insecticide as food bait. ANOVA on both experiments revealed significant differences between treatments, Bonferonni t-test, P>0.95. Means followed by different letters are significantly different. Additional support for the benefit of emitting ethyl acetate from traps containing Ferrolure+ and date fruit has been obtained in Oman by Abdullah and Al-Khatri (2005, Fig. 3).

Figure 3 shows the capture of *R. ferrugineus* in pheromone and date bits baited traps additionally emitting ethyl acetate in Oman (Adullah and Khatri, 2005). The experiment was conducted using ground traps. Some palm weevil trapping programs have recommended the use of traps without insecticide. We examined the efficiency of pheromone/food baited traps containing insecticide vs. those without insecticide. In Figure 4, it is obvious that addition of as little as 0.25% lannate to pheromone/food baited traps acts to retain arriving *R. palmarum*. In this experiment we found 10 *R. palmarum* in traps without insecticide. Of these 7, were alive suggesting that without insecticide *R. palmarum* still enter the traps but leave.

Figure 4 shows the capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane, ethyl acetate:ethanol (1:1) and insecticide. Insecticide was 1 L of 0.25% lannate or 1 L of water with no lannate. ANOVA (n=12-13) gave df=1, 23 F=15.07. Means topped by different letters are significantly different by Bonferonni t-test, P>0.95. This result is in agreement with weevil retention studies in which we demonstrated that without insecticide >90% *R. palmarum* escaped from a 20L plastic bucket after 24h (Oehlschlager et al., 1993b).

In the beginning part of this decade an elegant study sought to determine if the food component of palm weevil traps could be replaced by the chemical components that were responsible for the attraction generated by the fermenting food in palm weevil traps. In this study the antennally active common components of several highly attractive foods were chemically identified and then mixtures of these components were tested as a substitute for sugarcane in pheromone traps targeting *R. palmarum*. In the most promising of these experiments it was reported that a mixture of 28 components released in combination with ethyl acetate and ethanol was as effective as sugarcane in synergizing attraction of *R. palmarum* to pheromone-baited traps (Rochat et al., 2000). If this report is correct then trap servicing would be greatly simplified.

The original trapping of *R. palmarum* in traps baited with pheromone, blend H and containing lannate laced water were conducted in South America and showed that captures to traps baited in this way were as attractive as traps baited with pheromone, sugarcane and lannate laced water. When we repeated these experiments in Costa Rica with much attention to detail we obtained the results in Figure 5. Clearly blend H is not as attractive as sugarcane and in our experiments appeared to be repellent relative to ethyl acetate and ethyl acetate:ethanol. What is noteworthy in this study is that in week 3 traps baited with pheromone, ethyl acetate:ethanol and containing lannate laced water were as attractive as traps containing pheromone, sugarcane and lannate laced water. This indicates that for *R. palmarum* ethyl acetate\ethanol is beneficial in those situations where frequent servicing is not possible.

Figure 5 shows the capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane, ethyl acetate, ethanol and a blend of 28 components possibly attractive to *R. palmarum*. Mean (+SEM) *R. palmarum*/trap/week in traps baited with pheromone and different synergists. ANOVA (n=9-10) on log(X+1) transformed data gave Week 1: F=7.37; df=3,35; p<0.05; Week 2: F=6.19; df=3, 35; p<0.05; Week 3: F=8.16; df=3,35; p<0.05. Means topped by a different letter are significantly different, Bonferonni, P>0.95.

Replacement of food bait due to decomposition and desiccation is a major effort in trapping *R. palmarum* and *R. ferrugineus*. In the case of *R. palmarum* in the dry season food bait becomes dry and unattractive after 2 weeks while in the wet season decomposition renders food bait unattractive due to decomposition after 3-4 weeks. Attempts to construct artifical food bait from chemical odors (Rochat et al., 2000) have not been successful (Fig. 5). Water is an essential ingredient of traps since a primary method of retaining weevils in traps is for them to feed on insecticide-laden wet food. In

the Middle East where trapping is targeted against *R. ferrugineus* food bait in traps often dries out within a few days and traps loose their ability to retain attracted weevils.

We conducted several experiments to extend the useful life of trap food bait by addition of inexpensive materials that retard the evaporation of water, are not repellant to the weevils and are not toxic to humans. Figures 3 and 4 show typical results with one such additive, propylene glycol. Propylene glycol does not evaporate so traps containing it do not get dry. Propylene glycol is not toxic to humans (ethylene glycol, antifreeze, is toxic) and is relatively inexpensive. Propylene glycol prolongs the useful life of sugarcane baited traps until at least 7 weeks. In Figure 6 after 4 weeks traps with the propylene glycol are still more attractive than 2-week-old traps with water. Traps with propylene glycol were still attractive and contained liquid after 10 weeks.

Figure 6 shows the capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane and propylene glycol. Mean *R. palmarum* in pheromone traps containing commercial pheromone lures (ChemTica), sugarcane, lannate in water and variably propylene glycol. Treatments were traps baited additionally with fresh sugarcane in 750 ml of water containing 0.13% lannate (new sugarcane); 2-week-old sugarcane in 750 ml of water containing 0.13% lannate (2-week-old sugarcane); 6-week-old sugarcane in 750 ml of water containing 0.13% lannate (6-week-old sugarcane) and fresh sugarcane, ethyl acetate lures in 750 ml of water with 20% propylele glycol and 0.13% lannate placed November 14, 2000 (traps with propylene glycol). Ten traps of each treatment were placed. Means of capture are presented. ANOVA on data collected November 29 (n=9-11), December 16 (n=9-10) and January 14 (n=9-10) indicated no significant differences between treatments. ANOVA (n=8-10) on December 31 and February 1 (n=9-10) indicated traps containing new sugarcane were significantly more attractive than other treatments.

In Figure 7, traps prepared with propylene glycol remained attractive for 7 weeks. At this time point traps containing propylene glycol were still almost as attractive as freshly prepared traps.

Figure 7 shows the capture of *R. palmarum* in bucket traps containing pheromone, sugarcane and propylene glycol. The experiment was set up on January 14, 2001. All traps contained commercial pheromone lures (ChemTica). Treatments were traps baited additionally with fresh sugarcane in 500 ml of water containing 0.13% lannate (new sugarcane), 2-week-old sugarcane in 500 ml of water containing 0.13% lannate (2-week-old sugarcane), 6-week-old sugarcane in in 500 ml of water containing 0.13% lannate (6-week-old sugarcane) and fresh sugarcane, ethyl acetate lures in 750 ml of water with 50% propylene glycol and 0.13% lannate placed on January 14, 2001 (traps with propylene glycol). Ten traps of each treatment were placed. Means of capture are presented.

CONCLUSIONS

It can be concluded that emission of ethyl acetate from traps baited with the aggregation pheromone and suitable food more than doubles captures of *R. palmarum* and *R. ferrugineus* in traps. Substitution of chemical attractants for sugarcane in pheromone traps baited to attract *R. palmarum* results in very low captures. Addition of very low levels of insecticide (0.1%) to pheromone traps retains significant proportions of *R. palmarum* that would normally escape from these traps. Additon of propylene glycol to pheromone traps significantly increases attraction of *R. palmarum* after 2-3 weeks allowing servicing to be less frequent.

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Figures

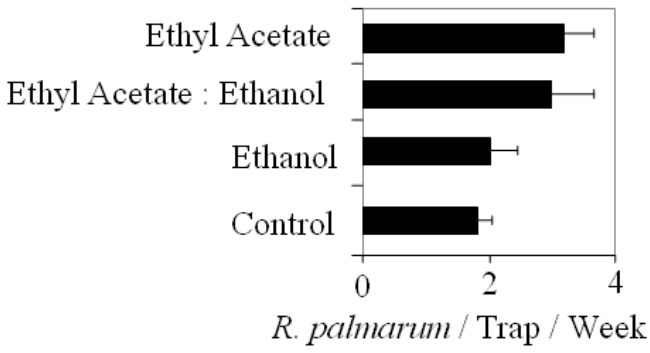

Fig. 1. *Rhynchophorus palmarum* capture in pheromone and sugarcane baited traps additionally emitting ethyl acetate and ethanol.

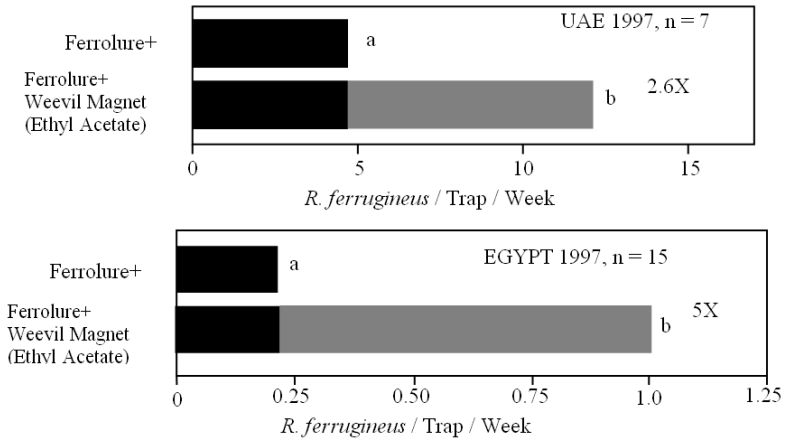

Fig. 2. Capture of *R. ferrugineus* in UAE and Egypt in pheromone and food traps additionally emitting ethyl acetate.

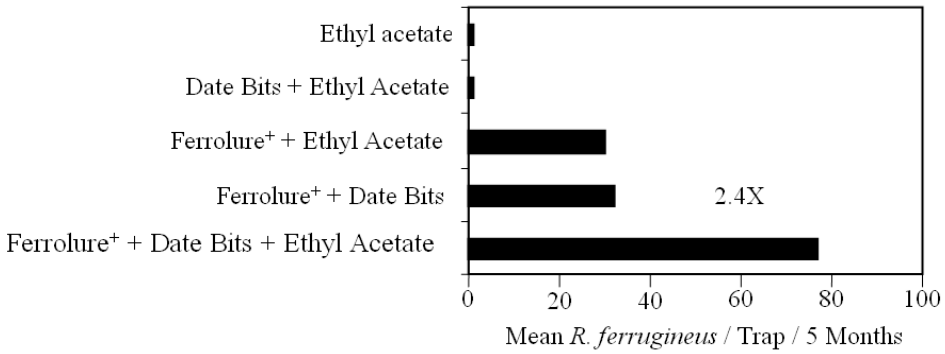

Fig. 3. Capture of *R. ferrugineus* in pheromone and date bits baited traps additionally emitting ethyl acetate in Oman.

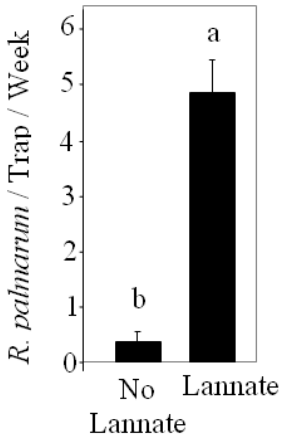

Fig. 4. Capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane, ethyl acetate:ethanol (1:1) and insecticide.

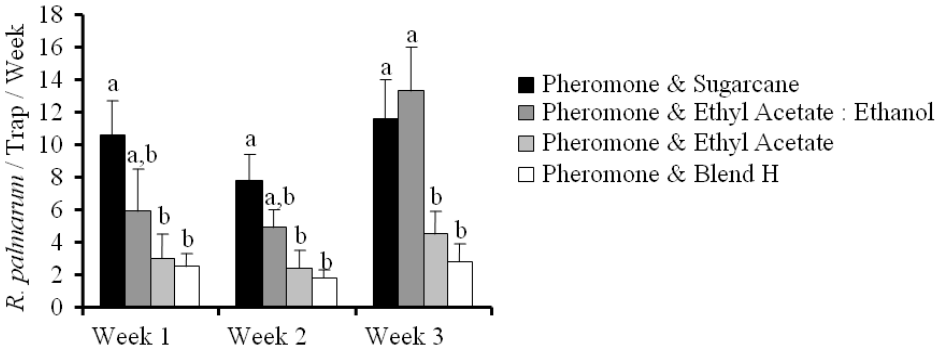

Fig. 5. Capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane, ethyl acetate, ethanol and a blend of 28 components possibly attractive to *R. palmarum*.

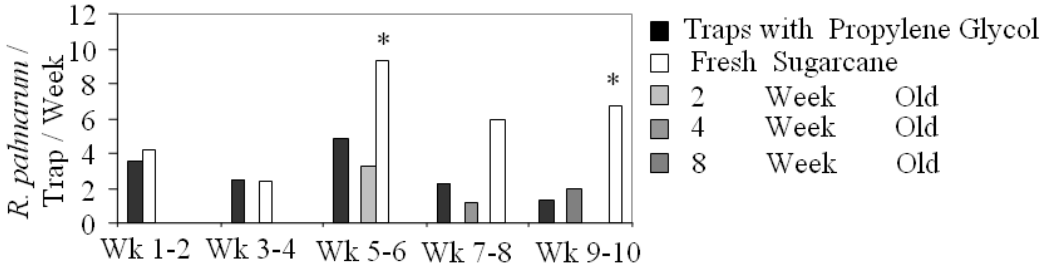

Fig. 6. Capture of *R. palmarum* in bucket traps baited with pheromone, sugarcane and propylene glycol.

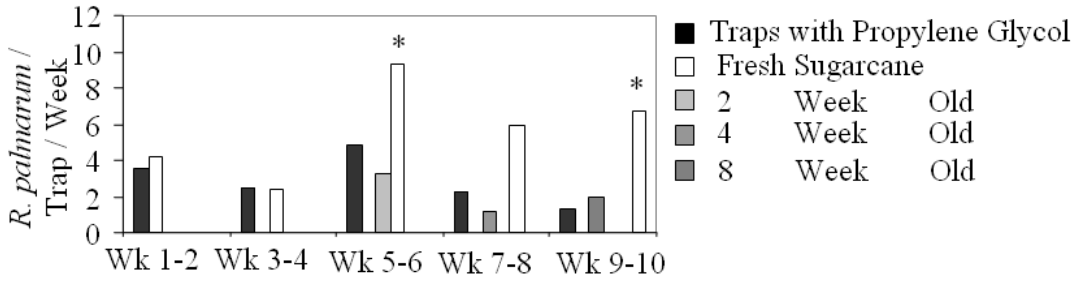

Fig. 7. Capture of *R. palmarum* in bucket traps containing pheromone, sugarcane and propylene glycol.

Semi-Field and Field Evaluation of the Role of Entomopathogenic Nematodes in the Biological Control of the Red Palm Weevil *Rhynchophorus ferrugineus*

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Keywords: *Phoenix dactylifera*, *Rhynchophorus ferrugineus*, *Steinernema*, *Heterorhabditis*.

Abstract

The objective of this study was to explore the efficiency of native isolates of entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* in controlling the population of the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) that attack date palm trees *Phoenix dactylifera* L. in the Arabic region. Semi-field studies showed that mortality in adults of the RPW infesting caged 5-year-old date palm trees reached 90 and 100% after 10 days of spraying the nematodes *Steinernema* and *Heterorhabditis*, respectively on and around the infested date palm trees. Increasing the dose from 2×10^6 to 4×10^6 nematode infective juveniles (IJ)/tree did not result in a significant increase in the pest mortality.

Field studies indicated that tested EPNs induced highly significant mortality in cocoons of the RPW aggregated in leaf petioles of 10-year-old date palm trees. Mortality in prepupae and pupae inside these cocoons were higher (up to 100%) than that in adult stage (up to 66.6%). The tested heterorhabditid isolates were more effective against RPW cocoons than the steinernematid ones. Regardless of the insect stage, *H. bacteriophora* induced 98.3% mortality in cocoons, followed by *H. indica* (90.41%), *Steinernema* sp. S1 (60.35%), while the least effective nematode was *Steinernema* sp. S2 (51.17%). These results support the possibility of using EPNs to prevent the emergence of adults from RPW cocoons at the beginning of spring and, in turn suppress the population density of RPW in the surrounding.

INTRODUCTION

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) is the most destructive pest of date palm trees *Phoenix dactylifera* L. in the Arabic region and Southern Europe. The unique agro-climatic conditions prevailing in the Middle East and the nature of the crop coupled with transportation of planting material have helped in the rapid spread of the pest in a short period of about a decade (Abraham et al., 1998). It invaded the United Arab Emirates in 1985, Saudi Arabia in 1987, Iran in 1990 and Egypt in 1993 (Murphy and Briscoe, 1999) and went west to Spain in 1994 (Barranco et al., 1996) and Italy in 2004 (Sacchetti et al., 2005). It also attacks other palm species like coconut, oil palms and *Washingtonia* palms (Kalshoven, 1950).

Adults of the RPW are attracted to palm trees and females lay 300 eggs during their 2-3 months-life cycle. The hatched larvae tunnel into the trunk or the terminal bud leading directly to the death of the tree (Griffith, 1987; Sivapragasam et al., 1990). Al Mohanna et al. (2000) reported that the pest can develop between 15 and 40°C. The larval

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stage has 12 instars and lasts mainly 60 days. On completion of their development, larvae move back to the base of the fronds where they pupate in elongated oval, cylindrical cocoons made out of fibrous strands. The complete life cycle of the weevil, from egg to adult emergence, takes an average of 82 days (OEPP/EPPO, 2008). The adult stage lasts up to 107 days. Despite the intensive efforts for controlling this pest, it is continuously spreading and destroying palm plantations. Because of the cryptic feeding habit of larvae chemical control has failed. Primary infestations always escape attention and symptoms may not become evident until extensive damage has already occurred. Management programs depend mainly on chemical insecticides (Girgis et al., 2002). Infested trees are usually injected with chemical insecticides. Chemicals may go through sandy soil to ground water and subsequently cause many environmental and health hazards. There is a strong emphasis on pheromone traps and biological control rather than insecticides (Murphy and Briscoe, 1999). Pheromone traps are usually used either for monitoring the population density of the weevil or for mass trapping the weevils.

Among several biological control agents entomopathogenic nematodes (EPNs) of the families *Heterorhabditidae* and *Steinernematidae* are the most promising. They have been isolated from different soil types, from sea level to high altitudes, and from natural habitats of disturbed ecosystems. The microscopic round worms are symbiotically associated with specific bacteria. The free-living infective stages (IJ) attack insects through natural openings and release the bacteria in the host blood, where they contribute to the death of the insect within 24-48 hours. Nematodes feed on host tissues and bacterial cells and complete their life cycle in 7-12 days. Huge numbers of IJ then emigrate from the insect cadaver looking for surviving victims. EPNs have a high reproductive potential, a broad host range and are easily applied with conventional spraying equipment. Development of large-scale mass-production technology (Ehlers, 2001) and easy-to-use formulations led to expanded use of EPNs in several countries (Grewal et al., 2002).

The objective of this study was adding evidence to the value of utilization of native isolates of EPNs in controlling the RPW through semi-field and field studies.

MATERIALS AND METHODS

Nematodes

Steinernema sp. S1, *Steinernema* sp. S2, *Heterorhabditis indica* HAS, and *H. bacteriophora* used in these studies are native isolates of EPNs extracted either from Qatif, Eastern Province, Saudi Arabia or Egypt. *Steinernema* sp. S1 was extracted from naturally infected adults of the RPW which were in turn collected by a pheromone trap in a date palm farm in the region. Identification of the extracted nematodes to the genus level depended on symptoms that appeared on nematode-infected *Galleria mellonella* larvae and the morphology of nematode developmental stages as described by Woodring and Kaya (1988), Poinar (1990) and Kaya and Stock (1997). *Heterorhabditis indica* HSA was isolated using the *Galleria* bait technique Bedding and Akurst (1975) and identified by CABI-Bioscience, England as mentioned in Saleh et al. (2001). *H. bacteriophora* was isolated and identified in Egypt (Shamseldean et al., 1996). All nematodes were maintained in the laboratory on larvae of *G. mellonella* according to Woodring and Kaya (1988).

Insects

Developmental stages of *R. ferrugineus* were collected from infested date palm trees in Qatif. Adults were collected from pheromone traps distributed in date palm farms. The collected insects were maintained in the laboratory on logs of soft wood-tissues of date palm trees until usage.

EXPERIMENTS

Semi-Field Studies

Date palm trees of 5 years old were transferred individually to halves of polyvinyl barrels, 80 cm diameter × 80 cm high, filled with sandy soil. Each tree was covered with a plastic screen cage (2 m high) to prevent escape of weevil adults. The cages were arranged out the laboratory building in Qatif during October when day the temperature was about 35±2°C. A total of 9 cages were used in this experiment (3 treatments × 3 replicates). Treatments were *Steinernema* sp. S2, *Heterorhabditis indica* HSA and control. The trees were artificially infested with adults of the red palm weevil at a rate of 10 weevils/tree/cage. After 24 hours, the water suspension of a specified nematode was applied on the basal part of the trees and soil around them at rates of 2 and 4 million infective juveniles/5 L of water/tree. Control plots received the same volume of water. The trees and the soil were inspected after 3, 5 and 8 days and insect mortality was recorded. Dead insects were transferred to White-traps for ensuring nematode development and propagation.

Field Studies

These experiments took place in date palm orchards infested with the RPW in Ismailia Governorate, Egypt during April when the day temperature was about 25°C. Water suspensions of four native nematode isolates (*Steinernema* sp. S1 (from Saudi Arabia), *Steinernema* sp. S2 (from Egypt), *Heterorhabditis bacteriophora* (from Egypt) and *Heterorhabditis indica* (from Saudi Arabia*)*) were prepared at a concentration of 2×10^6 IJ/L. Leaf-petioles of date palm trees were sprayed two successive times, 5 days-interval between them. Each tree received approximately 2 L of nematode suspension at each spray time. A 10-L back-sprayer was used in these experiments. Each treatment was replicated 5 times. Control treatments received the same volume of water. The nematode spray was conducted in the field just before sun set. After 5 days of the second spray, infestation sites in treated trees were dissected and RPW cocoons were collected and inspected for mortality. RPW stages inside these cocoons were classified to prepupae, pupae and adults. Dead insects were inspected again for ensuring nematode infection. Data were subjected to statistical analyses.

RESULTS

Semi-Field Studies

Data in Table 1 show that both Saudi nematodes *Steinernema* sp. S1 and *H. indica* HSA were efficient in controlling adults of RPW under semi-field conditions with differences according to the nematode, the duration after treatment and the used dose. Weevil mortality in date palm trees treated with the *Steinernema* sp. S1 when used in a dose of 2 million/IJ/tree recorded 33.3% after 3 days of treatment, 60% after 5 days, and 90% after 10 days of treatment. Increasing the dose to 4 million IJ/tree slightly increased the RPW mortality to 36.67, 63.33 and 90% after 3, 5 and 10 days of treatment, respectively. The heterorhabditid *H. indica* HSA looked more efficient than the steinernematid one. It recorded mortality of 60, 80 and 100% after 3, 5 and 10 days of treatment when used at 2 million IJs/ tree. When this nematode was used at 4 million/IJs/tree it caused mortality of 60, 86.67 and 100%, respectively. No RPW mortality was detected in control plots.

Field Studies

Cocoons of the RPW containing prepupae, pupae and new formed adults are frequently aggregate in leaf-petioles of infested date palm trees. Two successive sprays with EPNs of 5 days interval between them were applied to the leaf-petioles of the date palm trees in Ismailia and induced highly significant mortality in RPW cocoons (Table 2). Mortality of RPW stages in cocoons differed according to the used nematode as well as

the insect stage inside the cocoon. Prepupae and pupae in their cocoons were clearly more susceptible to nematode infection than the adult stage regardless of the used nematode. Mortality of prepupae and pupae inside cocoons ranged 50-100% while mortality of adults inside cocoons ranged 0-66.7%. The tested heterorhabditid nematodes were more effective against RPW cocoons than the steinernematid species. The most effective nematode was *H. bacteriophora* which induced 98.3% mortality in cocoons regardless the insect stag, followed by *H. indica* (90.41%) and *Steinernema* sp. S1 (60.35%) while the least effective nematode was *Steinernema* sp. S2 (51.17%). At the time of the experiment, the pupal stage formed 59% of total RPW individuals inside tested cocoons while prepupae and adults formed 21 and 20%, respectively.

DISCUSSION

In previous semi-field studies, Hanounik et al. (2000), reported that spraying date palm trees, 4-5-years-old, with the Saudi heterorhabditid nematode HSA-17 with the antidessicant "Leaf Shield" induced 87.5% mortality in adults of RPW compared to 65% mortality when the nematode was used with water only. Steinernematids were reported to be suitable for lower field temperatures while heterorhabditids were suitable for higher field temperature (El-Saadawy and Saleh, 1999). Shapiro et al. (2002) stated that choosing the right species against a particular pest in a particular environment is very important for successful biological control.

Achieving up to 98.3% mortality in RPW cocoons, the tested native nematode isolates are promising bioconrol agents against the pest. To our knowledge, this is the first field application study targeting the cocoons of RPW in leaf petioles of date palm trees. Targeting adults of the RPW, Abbas et al. (2000) stated that spraying the nematode *S. riobravae* on palm trees, at a rate of 8×10^6 IJs/tree in the United Arab Emirates caused 13.3% mortality in the RPW adults. However, application of nematodes in soil around tree trunk at a rate of 8×10^6 IJS/ tree gave 33.3-86.7% mortality. The relatively high proportion of RPW pupae inside cocoons probably came from the longer duration of pupal stage compared to other stages (prepuapae and adults) inside cocoons. Al-Mohanna et al. (2000) mentioned that cocoons were constructed within 5 days and the period between the beginning of pupation and the emergence of adults ranged from 13 to 24 days. Encouraging results of our semi-field and field studies support the possibility of using EPNs to prevent the emergence of adults from RPW cocoons at the beginning of spring and, in turn suppress the population density of RPW in the surrounding.

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Tables

Table 1. Mortality in adults of *Rhynchophorus ferrugineus* in date palm trees after application of *Steinernema* sp. S1 and *Heterorhabditis indica* HSA (cage experiment).

Nematode	Dose (IJ/tree)	% Mortality {Mean (SE)}		
		3 days	5 days	10 days
Steinernema sp. S1	2 million	33.33 (3.33)	60 (5.77)	90 (5.77)
	4 million	36.67 (8.82)	63.33 (8.82)	90 (5.77)
Heterorhabditis indica HSA	2 million	60 (5.77)	80 (5.77)	100
	4 million	60 (11.54)	86.67 (8.82)	100

Table 2. Mortality in cocoons of *Rhynchophorus ferrugineus* infesting date palm trees after spraying with entomopathogenic nematodes.

Nematode	Insect stage inside cocoons							
	Prepupae		Pupae		Adults		Total	
	n	% mortality	n	% mortality	n	% mortality	n	% mortality
Steinernema sp. S1	10	90	22	63.63	9	22.22	41	60.35 c
Steinernema sp. S2	15	86.66	20	50	10	10	45	51.17 c
Heterorhabditis bacteriophora	9	100	33	100	3	66.67	46	98.33 a
Heterorhabditis indica	10	90	37	100	4	0	51	90.41 b
Control	2	0	20	20	22	0	44	8.33 d

Means followed by different letters are significantly different (*P>0.001*).

First Record of the Red Palm Weevil [*Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*)] in Libya

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Abstract

The red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*) is an economically important, tissue-boring pest of date palm in many parts of the world. In Libya, adults of *R. ferrugineus* were collected occasionally from Tobruk city during January 2009. In May 2009, 40 pheromone-keromone traps were introduced by the Pest Control Center, Libyan Agriculture Ministry; 19 traps were placed at 19 smallholder farms inside Tobruk city; 14 traps were placed at 14 smallholder farms outside the city (more than 15 km from the city center). Seven traps were placed at 7 commercial farms at El-Gaghboob oasis (300 km south Tobruk) to detect and monitor the red palm weevil. Data revealed that the occurrence of adults was in the center of Tobruk. The mean recorded numbers ranged between 0.33-7.91 adults/trap/week. The total numbers of collected adults was 565 during the investigation period. Traps placed at Tobruk borders and El-Gaghboob oasis did not record any attracted adults. Detection and monitoring of red palm weevil should be undertaken in other places in Libya because it invaded many close countries.

INTRODUCTION

The date palm, *Phoenix dactylifera* (*Palmae*) is the most common and widely cultivated plant in the arid regions of the Middle East and North Africa where, in many areas, its fruit has provided the staple carbohydrate food of local people for nearly 5000 years (Purseglove, 1972; Jones, 1995; Murphy and Briscoe, 1999).

The red palm weevil, *Rhynchophorus ferrugineus* Oliv., (RPW) (*Coleoptera: Curculionidae*) (Figs.1-4), is an economically important, tissue-boring pest of date palm in many parts of the world. The date palm crop in these countries is now under threat. In the mid 1980s, it was discovered attacking palms in the Arabian peninsula (Gush, 1997; Abraham et al., 1998). It was recorded for the first time in the United Arab Emirates in 1986; it was then found in Saudi Arabia in 1987 and in the Islamic Republic of Iran in 1992. However, it has now crossed the Red Sea into North Africa, as the latest record is from the Sharqiya region of Egypt (Cox, 1993). By 1995, it had infested over 10,000 farms across Arabia. In infested plantations, yields have been estimated to have dropped from 10 tonnes to 0.7 tonnes per hectare (Gush, 1997).

Females lay about 300 eggs in separate holes. These eggs are creamy white, oval in shape about 2.6 mm long to 1.1 mm wide. The eggs hatch in 2 to 5 days into legless grubs which bore into the interior of the palm and feed on the soft tissues of the palm. The length of the full grown larva is 50 mm and the width is 20 mm. The larval period varies between 1-3 months. Then a pupa is formed (cocoon) and at the end of the pupation period the adult emerges.(Figs. 1-5).

The weevils are attracted to dying or damaged parts of palms but it is possible that undamaged palms are also attacked. The males of *R. ferrugineus* produce a pheromone which causes the weevils to aggregate on damaged trees (Gunawardena and Bandarage, 1995). The larvae can only bore in soft tissue; for example, in the tree crown, upper part of the trunk and at the base of petioles. They can also bore into the trunk of young palms

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and the decaying tissue of dying palms.

Aggregation pheromones have been reported as effective tools for monitoring and trapping *R. ferrugineus* in the field (Gunnawardena and Badarage, 1995a,b; El-Garhy, 1996; El-Ezaby et al., 1998).

In Libya, one adult of *R. ferrugineus* was collected occasionally from Tobruk city (North east of Libya) during January 2009. The sample was preserved in the laboratory to confirm identification. The aim of the present investigation is to study the pest status of the red palm weevil at Tobruk region and El-Gaghboob oasis.

MATERIALS AND METHODS

In May 2009, 40 pheromone-keromone traps were introduced by the Pest Control Center, Libyan Agriculture Ministry. 19 traps were placed at 19 smallholder farms inside Tobruk city while 14 traps were placed at 14 smallholder farms outside the city (more than 15 km from the city center). On the other hand, 7 traps were placed in 7 commercial farms at El-Gaghboob oasis (300 km south of Tobruk) to detect and monitor the red palm weevil. The distance between each two traps was between 800-1000 m inside the city.

The standard pheromone-keromone trap (Fig. 5) used in this study consisted of (i) a 10-L yellow plastic container; the container had two 10 cm diameter openings for the entrance of the attracted adults, (ii) soft date fruits were placed at the lower part of the container as baits with 2 L tap water. One ml detergent was added to the mixture to kill the trapped adults. Pheromone and keromone packages were hung up by a metal wire on the inner side of the container cover.

The commercially registered pheromone lure was used under the trade name PO28 Ferrolure+, 700 mg lure (ChemTica International S.A., Costa Rica). The components of this pheromone lure are 4-methyl-5-nonanol (9 parts) + 4- methyl-5 nonanone (1 part) - purity of both components >95% release rate 3-10 mg/day. Minimum 700 mg/lure total mixture. Keromone used in this study is ethyl acetate 95% mixed with jelly as release substance. One package contained 50 ml.

Pheromone-keromone traps were placed on the ground with the lower half of the trap inserted in the ground between the date palm trees. Traps located at ground level captured significantly more weevils than those hung up above the ground (Oehlschlager et al., 1993). Traps were examined weekly and numbers of caught attracted adults were recorded.

RESULS AND DISCUSSION

Tobruk is a coastal city on the Mediterranean basin. The vegetation cover in the city is wild vegetation spots, smallholder farms and plantations in the house backyards. Date palm is one of the most popular trees in these areas. The first detection of the red palm weevil was in an area characterized by a raise of the subterranean water level in the center of Tobruk. This detection was in January 2009.

Table 1 shows the numbers of *R. ferrugineus* per trap per week inside Tobruk city during the summer of 2009. Data revealed that the mean recorded numbers ranged between 0.33-7.91 adults/trap/week. The total numbers of collected adults was 565 during the investigation period. However, no symptoms or advanced infestations were observed on the palm trees grown in insect occurrence locations. This agrees with Bokhari and Abuzuhairah (1992) who reported that because the red palm weevil is a concealed tissue borer, symptoms of attack at an early stage of infestation are difficult to detect. Later in the infestation process the presence of larvae can be detected through the occurrence of tunnels on the trunk and at the bases of leaf petioles, and through the presence of frass and plant sap which oozes from these tunnels. When a palm is severely infested, the stem or crown sometimes breaks off the tree (Abraham et al., 1998). On the other hand, traps placed at Tobruk borders and EL-Gaghboob oasis did not record any attracted adults. In this concern, El-Garhy (1996) reported that catch rates were highest during the period from April to June (50-65 weevils). Also, El-Ezaby et al. (1998) reported maximum catches in March and April which corresponds to the warmer weather in Egypt.

Palm trees in wild vegetations are often out of regular control procedures, therefore they are considered as "natural reservoirs" which release adults of *R. ferrugineus* from time to time to threaten new areas. Programmers should put in mind this situation during the planning of "the national campaign of monitoring and eradication of red palm weevil". The general situation of obtained data in the present study indicate the possibility of promising eradication of this invaded pest in Tobruk in the current time. Furthermore, countries where occurrence of this pest has been reported must take additional emergency measures to limit its spread and eradicate the invaded pest. It was possible by the establishment of demarcated areas which include marking infested zones and buffer zones and with the adoption of chemical and other measures. Generally, detection and monitoring of red palm weevil should be undertaken in other places in Libya because it invaded many close countries.

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Table

Table 1. Mean ± SD numbers of *Rhynchophorus ferrugineus* adults/trap/week in different locations inside Tubrouk city during the season of 2009.

Trap No.	May		June				July			August		September		October			November
	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	1st week	2nd week	1st week	2nd week	1st week	2nd week	3rd week	1st week
1	3	5	2	0	0	0	13	3	6	1	5	7	10	-	12	11	-
2	2	8	2	1	0	2	5	4	4	3	4	8	9	-	3	2	-
3	1	7	0	1	1	0	3	0	2	1	7	2	7	-	5	6	-
4	3	1	1	0	-	-	0	0	0	0	2	0	13	-	10	15	-
5	0	0	-	-	-	-	10	4	7	7	9	17	1	-	12	1	-
6	0	2	-	-	-	1	2	2	2	2	2	5	-	1	1	0	-
7	-	-	-	-	-	-	-	-	-	-	2	2	-	3	4	-	1
8	-	-	-	-	-	-	-	-	-	-	1	0	-	8	-	-	-
9	-	-	-	-	-	-	-	-	-	-	1	3	-	10	9	-	16
10	-	-	-	-	-	-	-	-	-	-	2	25	-	4	4	-	13
11	-	-	-	-	-	-	-	-	-	-	5	5	-	5	0	-	-
12	-	-	-	-	-	-	-	-	-	-	-	6	-	9	0	-	27
13	-	-	-	-	-	-	-	-	-	-	-	2	-	8	1	-	2
14	-	-	-	-	-	-	-	-	-	-	-	16	-	0	8	-	7
15	-	-	-	-	-	-	-	-	-	-	-	0	-	0	1	-	2
16	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4	-	1
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	0
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	13
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	5
Total	9	23	5	2	1	3	33	13	21	14	40	99	45	48	87	35	87
Mean ± SD	1.50 ± 1.12	3.80 ± 1.74	1.25 ± 2.91	0.50 ± 0.71	0.33 ± 0.69	0.75 ± 0.91	5.50 ± 2.14	2.17 ± 1.30	3.50 ± 1.56	2.33 ± 2.29	3.64 ± 1.58	6.19 ± 2.64	7.50 ± 1.95	4.00 ± 1.90	5.44 ± 1.99	5.80 ± 2.35	7.91 ± 2.84

Figures

Fig. 1. Eggs of *R. ferrugineus.*

Fig. 2. Larva of of *R. ferrugineus.*

Fig. 3. Adult of *R. ferrugineus* emerged from cocoon.

Fig. 4. Adult stage of red palm weevil *R. ferrugineus* found in Tobruk city.

Fig. 5. Pheromone-keromone trap of red palm weevil *R. ferrugineus.*

Effect of Red Palm Weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) Aggregation Pheromone Traps' Height and Colors on the Number of Captured Weevils

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Keywords: *Rhynchophorus ferrugineus*, pheromone traps, height, color

Abstract

Red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) is controled using Integrated Pest Management, which depends on aggregation pheromone traps. Field trials were conducted on date palm plantations of Al Rahba in Abu Dhabi (UAE) during 20/11/2008-30/7/2009 containing 5 replications and 8 treatments (pheromone trap colors and height). The traps were hung on four different heights on the palm tree trunks (ground level, 0.5, 1.0 and 1.5 m for each color) and the aggregation pheromone 4-Methyl-5-Nonanol 90% + 4-Methyl-5-Nonanol 10% (700 mg), 350 gram fodder dates fruit in addition to about 5 L water were added to each trap to study the effect of traps' height on the number of captured weevils. Results indicated that the total number of captured weevils was 5487, the numbers of caught weevils for per color was 3360 and 218 weevils for red and white traps respectively, and the numbers of catches per height were 1367, 1683, 1335, and 1093 weevils for four heights (ground level, 0.5, 1.0 and 1.5 m) respectively. The highest number of catches (1063) was in red traps hung on 50 cm height. This treatment dominated over the other seven treatments. The lowestnumber of catches, 462 weevils, was recorded in white traps hung on 1.5 m. There are no significant differences between red traps which were put on land service, which caught 859 weevils, and on 1.0 m height, with 798 catches, and these two treatments dominated over the other five treatments. There are no significant differences between red traps on 1.5 m, catching 638 weevils and white traps on 0.5 m, catching 620 weevils. These two treatments dominated over white traps put on land service and caught 508 weevils. There were no significant differences between other treatments. These results indicate the importance of aggregation pheromone traps in controlling red palm weevil and the necessity of using red traps and hanging the traps on 0.5 m height on the palm tree trunk. Study and research should be continued to improve the effectiveness of this technique.

INTRODUCTION

Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) is one of the most destructive pests of coconut (*Cocos nucifera* L.) in South and Southeast Asia (Sivapragasam et al., 1990; Sadakathulla, 1991). It is a concealed tissue borer (Faleiro et al., 1998; Faleiro and Satarkar, 2003; Faleiro and Satarkar, 2003a), it is of major economic importance as the insect attacks palm trees all over the Gulf Countries now (Bokhari and Abuzuhari, 1992; Abraham et al., 1998, 2000, 2002; Al-Saoud, 2006, 2008; Al-Saoud et al., 2010). Since the mid 1980s, (RPW) has caused severe damage to date palm trees in several Middle Eastern countries (Abozuhairah et al., 1996). It is relevant to point out that this insect was first reported in the Indian Museum Notes in 1891 while Lefroy (1969) first described it as a pest of coconut in India. It was first described as a serious pest of date palm by Madan Mohan Lal (1917) in the Punjab from India. Buxton (1920) found that this pest caused serious

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damage to date palm in Mesopotamia (Iraq).

The pest is difficult to control in the early stage of attack because it is an internal tissue borer (Abraham et al., 1998) with the synthesis of the male produced aggregation pheromone "Ferrugineol" (4-methyl-5-nonanol) (Hallett et al. 1993). The food bait and pheromone act synergistically to attract adults of RPW (Abraham et al., 1989; Hallett et al., 1999; Abraham et al.,1989, 2000; Vidyasagar et al., 2000; Nair et al., 2000; Al-Saoud, 2007, 2008; Al-Saoud et al., 2010).

Aggregation pheromone traps are the main component of any Integrated Pest Management programme, they attract both male and female weevils (Abraham et al., 1998, 2001; Oehlschlacher et al., 1998, 2002; Al-Saoud, 2006, 2007, 2008, 2009a; Al-Saoud et al., 2010). In the Middle East as well as in India the sex ratio of weevil captures was reported to be female dominated in these traps (Abraham et al., 1999; Faleiro, 2000; Faleiro and Rangnekar, 2000; Faleiro and Satarkar, 2003a; Al-Saoud, 2004, 2006, 2009; Al-Saoud et al., 2010). It is a desirable factor that aggregation pheromone traps attract and capture more female weevils, which lay eggs. Killing this captured male and female weevils, the traps play a significant role in reducting the population of this pest.

The weather conditions affected the number of captured red palm weevils. Abraham et al. (1998) reported that the pheromone traps caught a huge numbers of weevils during March-May and, September-November, and the weevils captured dropped significantly during the height of summer or winter seasons. Faleiro and Rangnekar (2001) and Faleiro (2004) reported that red palm weevil pheromone traps caught large weevil numbers between October-November, while weevil activity is least between June-July, under costal humidity conditions of Western India. Al-Saoud (2004, 2006, 2007, 2009) reported the peak of activity during March-May in Al-Rahba (UAE). The female dominated in weevil captures in aggregation pheromone traps (Abraham et al., 1999; Faleiro and Chellapan, 1999; Faleiro and Rangnekar, 2000; Vidyasagar et al., 2000; Al-Saoud, 2004, 2006, 2008, 2009; Al-Saoud et al., 2010).

Initial attempts to control red palm weevil, a major pest of the date palm in the Kingdom, with insecticides were not successful (Bokhari and Abozuhairah, 1992) and since 1994 an Integrated Pest Management (IPM) strategy, modeled on the lines of tackling the pest on coconut in India was implemented in the Kingdom. This IPM strategy has successfully suppressed the pest in the date plantations of Saudi Arabia (Abraham et al., 1998).

The use of pheromone traps was the major component of the IPM strategy, used to capture and kill the flatting weevil population.

In any insect trapping program, it is desirable to capture a higher number of females, as the adult female contributes directly to the population build up by laying eggs.

In nature, male to female population was reported as 1.32:1 (Nirula, 1956), while (Abraham et al., 1999) found in Arabia Saudi Kingdom that the weevil captures in different operational areas between mid 1994 to December, 1997 varied from 1:2.35 to 1:3.06, with an overall average of 1:2.68 in favour of females, while (Al-Saoud, 2006) found this ratio 1:1.33-1:2.2. Al-Saoud (2007) found that the sex ratio was 1:1.56. Al-Saoud (2009) recorded the sex ratio as 1:1.89. While as the captures were female dominated, pheromone trapping along with other components of the IPM strategy contributed in suppressing the build up of the pest. This is supported by the fact that the total number of weevil trapped during 1997 was reduced to 3806 as compared to 5308 and 5533 weevils captured in Al-Hassa during 1995 and 1996, respectively. In this context it is relevant to point out that Oehschlager et al. (1995) obtained over 90% reduction in weevil captures of *R. palmarum* after two years of pheromone trapping in oil palm plantations of Costa Rica. These captures were female dominated too.

Pheromone trapping of red palm weevil is therefore an ecologically safe and environmentally friendly tool in the IPM strategy currently adopted worldwide for red palm weevil management in date palm plantation areas. This powerful component of the IPM program can be implemented on a large scale either by the state or by farmers on a collective basis.

The trap effectiveness is affected by many factors, colors (Hallett et al., 1999; Abdallah and Al-Khatri, 2005; Al-Saoud et al., 2010), pheromone type, trap contents, food bait, and trap sites (Hallett et al., 1999; Faleiro, 2004; Al-Saoud 2008a). The pheromone trap height affects the effectiveness of the traps. Faleiro (2004) and Al-Saoud (2008) found that the height of 1 m on the date palm trunk from the ground registered the best weevil captures.

The purpose of this study is to evaluate the effect of trap site on trap effectiveness in date palm plantations in Al-Rahba (Abu Dhabi) through captures of RPW adults using pheromone traps.

MATERIALS AND METHODS

The experiment was conducted at five farms at Al-Rahba, Abu Dhabi (UAE) during 20 December 2008 - 30 July 2009. Each farm contained about 140 date palm trees of different ages (6-25 years).

40 food baited pheromone traps were set throughout Al-Rahba, date palm plantations where no IPM practices for weevil management were followed to monitor the activity of RPW in these plantations.

Traps

Pheromone traps were fabricated using about 8-L capacity high density polyethylene (HDPE) bucket with four windows (1.5×1.5 cm) cut equidistantly 4 cm below the upper rim of the bucket. The distance between each window and the bottom of the bucket was 16 cm. The bucket was covered with a lid that had three windows similar to the those on the sides. The upper surface of the lid had a small handle to ease opining the trap and the lower side had a small knob on which a wire was fixed to hold the pheromone and kairomone dispensers. The outer surface of the bucket was rough with small projection (1-2 mm) to help the weevils climbing to the trap and enter. Each trap contained, 350 g of dates (the date fruits used were forage fruits or those that had dropped around palm trees in the farms, and these fruits are not consumable and incur no cost, according to the farmers), a dispenser of the *R. ferrugineus* male aggregation pheromone containing 700 mg of the active ingredient (4-Methyl-5-Nonanol(9 parts)+ 4-Methyl-5-Nonanol(one part) at 95% purity, was hung on the inner side of the bucket lid with a piece of wire, and about 5 L of water, with a water level inside of 4-5 cm, which was lower on the side of the opening of the bucket. Serial numbers were assigned to each trap and locations were numbered from 1 to 8 on every farm. The water was always replenished to keep sufficient moisture in each trap. Food bait (dates) was changed monthly. The pheromone was added every 45 days during the cold period (December-May) and every month during the warm period (May-July). The number of weevils captured (male, female and total) were recorded weekly, and the trap content was shaken well to prevent growing any fungi or anything. The traps were shifted to a new location after weekly results had been taken, to avoid a location effect, and to know the insect numbers in each location and in each treatment on every farm during the study period. Maintenance was done continuously.

Trap Colors

The trap colors were white which is commonly used in (UAE) and red to compare the effectiveness.

Experimental Design and Trap Installation

The experimental design was a randomized complete block design with nine treatments (trap height on the palm tree trunks, which were ground level, 0.5, 1.01 and 1.5 m height, for each color) and five replicates (farms). A total of 40 traps were installed for a trapping period from 20/11/2008 until 30/7/2009. This time period (253 days) was selected because it includes the parts of the season in which the adults of red palm weevil are more active and reach their population peak. A large beetle population in the field

amplifies the effect of trap set and color on weevil catch. The distance between traps was about 50 m. Traps were fixed to the date trees trunks with nylon wires. Captured weevils were collected weekly. The traps were surveyed at weekly intervals, when the results were recorded (number of males, females and total weevils, weekly, monthly and cumulative numbers during the studying period), in each trap and farm. Every trap was shifted to a new location after weekly results had been taken, to avoid a location effect, and to know the insect numbers in every location and in every treatment on each farm during the study period.

A monthly record of the number of weevils trapped in the 40 (N) pheromone traps was maintained for the period of December 2008 till July 2009 to see the activity of red palm weevil during the different months of the studying period.

The data were processed and subjected to ANOVA test.

RESULTS AND DISCUSSION

Monthly RPW Activity in Al-Rahba during the Studying Period

The weevil is found throughout the whole studying period in the date palm area in Al-Rahba (Fig. 1). The number of caught weevils/trap/month was different from one month to another. The rate of captures was 8.4, 4.8, 13.5, 33.5, 24.6, 15.5, 12.0 and 10.3 weevil/trap/month for December, 2008, January, February, March, April, May, June, and July, 2009, respectively. The highest catch was found during March and April, and the lowest was in December and January. Similar results were found by Abraham et al. (1999), Vidhyasagar et al. (2000), Al Saoud (2004, 2006, 2009) and Al-Saoud et al. (2010). While in Saudi Arabia, Abraham et al. (1999) found high weevil's activity in April to November, 1995, but in 1996 he got two peaks of activity - one in May to June and the other in October. But in 1997, the two peaks were found in May and September.

Consequently, reproduction of the insect occurs all year, and the damage increases, and control is difficult to achieve, especially, using chemicals, because application of pesticides must be done during mid February till the end of March (pollination period), and from the beginning until the end of the maturing and harvesting of the crop.

Effect of Red Palm Weevil (*R. ferrugineus*) Aggregation Pheromone Trap's Height on the Number of Caught Weevils

The study shows that there were differences in caught weevils in different heights of traps (Fig. 2). And the number of captures was found 1367, 1683, 1337 and 1100 weevil on the ground level, 0.5, 1, and 1.5 m traps' height respectively. The analysis results showed that 0.5 m trap's height on the date palm trunks was dominant over other treatments. This result nullified the results of Faleiro (2004) in India where he got the best results at 1 m height on the trunk of date palm and coconut.

Al-Saoud (2004, 2006, 2007) recommended putting traps 3 m far from the date palm and 12 cm deep in the sand. This is to avoid the infestation in the date palm. Al-Saoud (2008) found best results at 1 m height on the date palm compared to traps put at 1 m height on seeder and 3 m far from date palms in 7 and 12 cm depth in the sand. These results are partly supported by Hallett et al. (1999), who found the best results for ground level traps compared to 2 m and higher.

Effect of Red Palm Weevil (*R. ferrugineus*) Aggregation Pheromone Trap's Colors on the Number of Caught Weevils

The number of caught weevils is variable in two colors. The red trap catches 3358 compared to 2129 weevils in the white trap (Fig. 3).

The same result was obtained by Abdallah1 and Al-Khatri (2005) in the Sultanate of Oman. Al-Saoud et al. (2010) in the United Arab Emirates, found that the red trap color is dominant to catch the weevil over the white color, which is commonly used in the UAE, catching the lowest number of weevils. Falerio (2005) shows no significant

difference in the number of weevil caught in different colors.

Sex Ratio of *R. ferrugineus* Caught in Aggregation Pheromone Traps

The results revealed that the total number of caught weevils was 5487 af which 1757 were male and 3712 were female showing a sex ratio of 1:2.12.

This result differs from the results obtained by Abraham et al. (1999) who showed that the weevil captures in different operational areas between mid 1994 to December 1997 varied from 1:2.35 to 1:3.06, with an overall average of 1:2.68 in favour of females. These results are reported by other workers (Al-Saoud, 2006, 2008, 2009), who got the sex ratio between 1:1.33 to 1:2.28. But, this present study showed the female number increasing due to lack of a suitable control method and reproduction of the insect.

The Number of *R. ferrugineus* Males Caught in Aggregation Pheromone Traps

The numbers of caught male weevils in 8 different treatments were 250, 172, 328, 199, 250, 180, 205, and 173, respectively (Fig. 4). The statistical analysis revealed that the red color on 0.5 m height caught maximum weevil numbers and dominated over the other 7 treatments. There is no significant difference between red traps on ground level and 1 m height, but these 2 treatments dominated over the others and also no significant difference was available in different heights of white traps. Similar results were found by Al-Saoud et al. (2010).

The Number of *R. ferrugineus* Females Caught in Aggregation Pheromone Traps

The female numbers caught are variable in different treatments. It was 609, 336, 735, 421, 548, 357, 435, and 280 females, for these 8 treatments respectively. Statistical analysis showed the dominance of the red color on 0.5 m height over all other treatments. There are no significant differences between the red color on ground level and 1 m height. The red trap on ground level dominated over other treatments. There are no significant differences between the red color on 1.5 m high and the white color on ground level, 1 and 1.5 m heights. The white color on 0.5 m. high dominated over the white color on 1.5 m. There are no significant differences between other treatments. Al-Saoud et al. (2010) found similar results in Al-Rahba.

The Total Number of *R. ferrugineus* Caught in Aggregation Pheromone Traps

The total numbers of caught weevils were 859, 508, 1063, 620, 798, 539, 638 and 462 for these 8 treatments respectively. The statistical analysis showed that the red color trap on 50 cm height dominated over the other 7 treatments, and there are no significant differences between the red color on ground level and 1 m height, and these two treatments dominated over other treatments. There are no significant differences between the red color on 1.5 m height and the white color on 0.5 m height, and these two treatments dominated over white traps on ground level and 1.5 m height. There are no significant differences between other treatments. These results differ from the recommendations of Al-Saoud (2004, 2006, 2009) that indicated to place the trap 3-4 m far from palm trees, and fixed in a hole of 12-15 cm depth in the sand. It agrees with the results of Faleiro (2004) and Al-Saoud (2008a) who found that the trap height of 1 m from the ground on the palm tree trunk recorded the best weevil captures, compared with other treatments, while the results indicated that the 0.5 m height recorded the best captures.

These results indicate the importance of the use of a suitable color for red palm weevil aggregation pheromone traps, and sets these traps on 0.5 m on the date palm trees trunks in infested plantation areas, all over the year, and these traps should be maintained regularly and new pheromone added. The food dates and water should be changed as when required. Deep study is needed to get best results of pheromone traps and to improve these techniques, because it is very useful and safe for thee environment and human beings.

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Figures

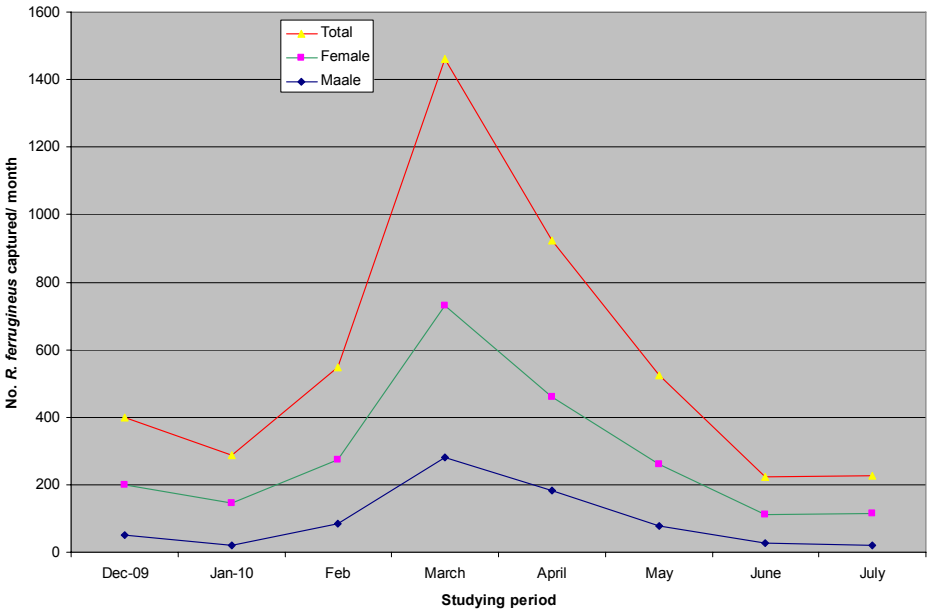

Fig. 1. Activity of *R. ferrugineus* at Al-Rahba(Abu Dhabi) during December 2008 to July 2009.

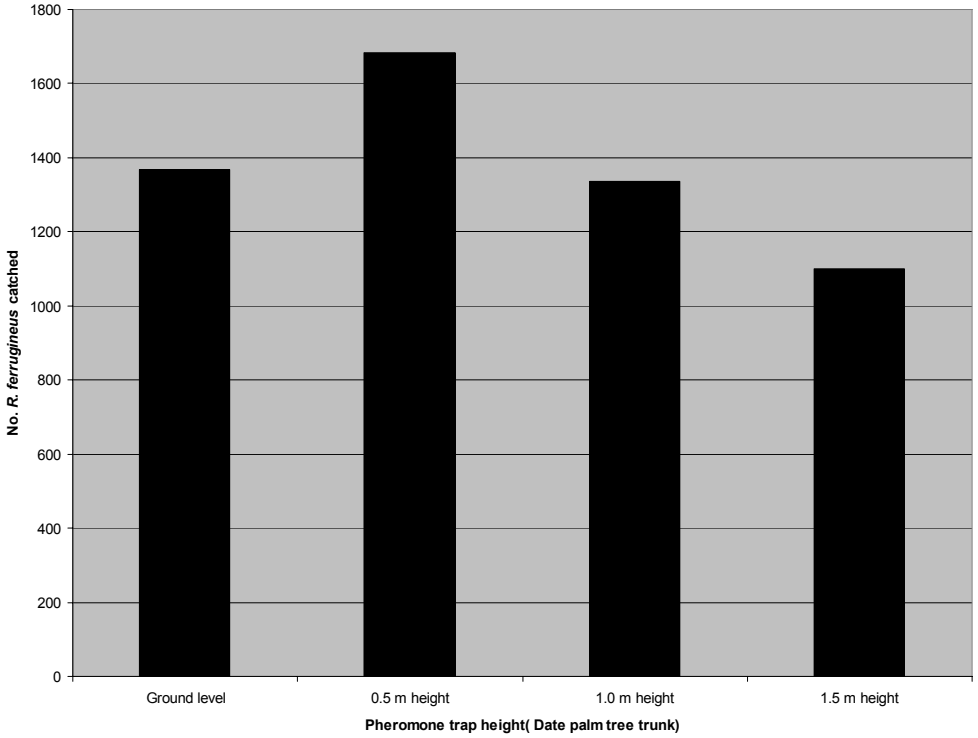

Fig. 2. Effect of *R. ferrugineus* Oliv. aggregation pheromone traps' height on the total caught weevils at Al-Rahba (Abu Dhabi) during 20 December 2008 to 30 July 2009.

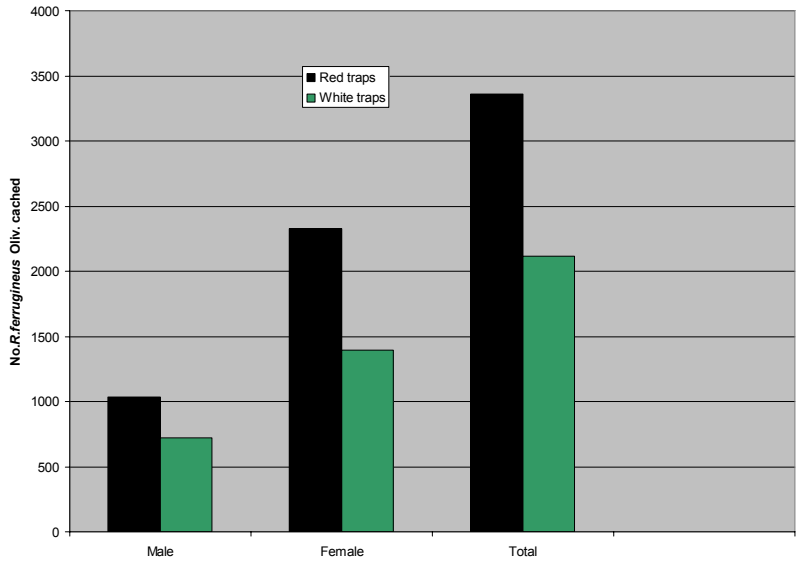

Fig. 3. Effect of *R. ferrugineus* Oliv. aggregation pheromone traps color on the number of males, females and total caught weevils at Al-Rahba (Abu Dhabi) during 20 December 2008 to 30 July 2009.

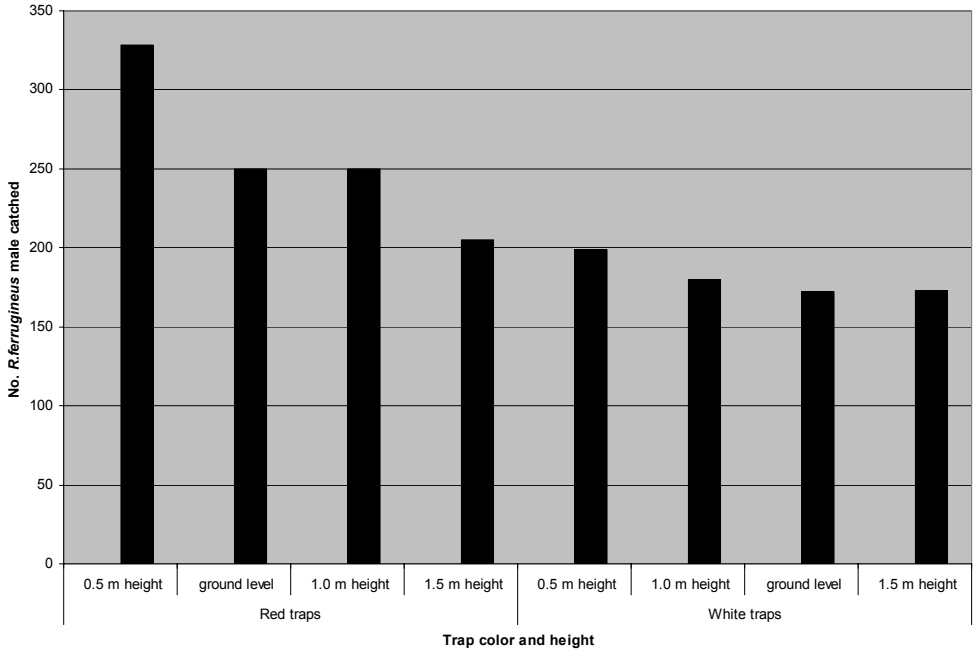

Fig. 4. Effect of *R. ferrugineus* Oliv. aggregation pheromone traps' color on the number of male weevils caught at Al-Rahba (Abu Dhabi) during 20 December 2008 to 30 July 2009.

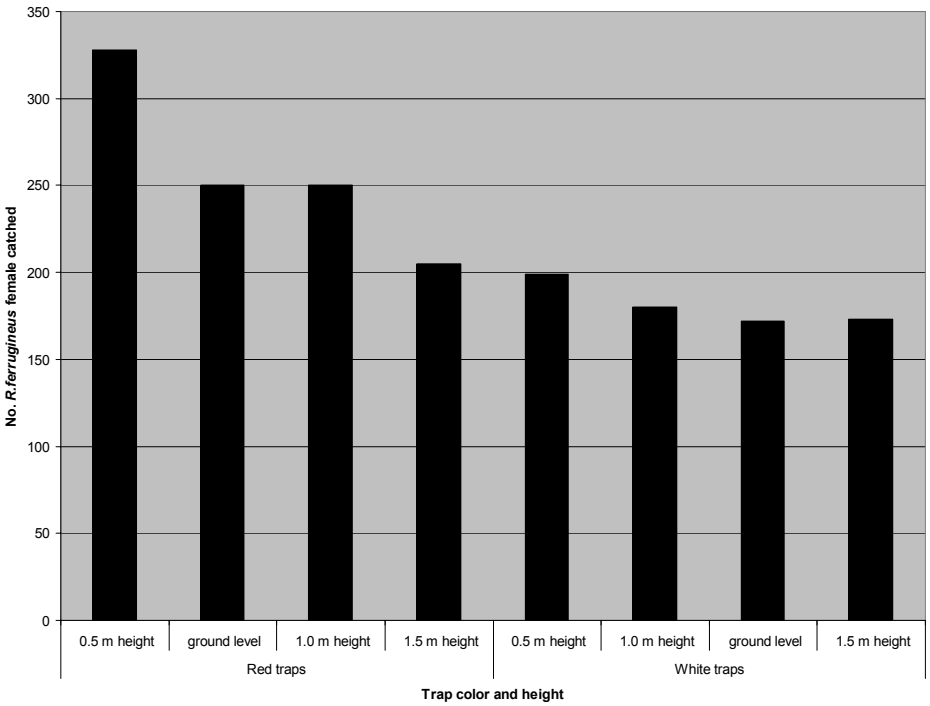

Fig. 5. Effect of *R. ferrugineus* Oliv. aggregation pheromone traps color on the number of female weevils caught at Al-Rahba (Abu Dhabi) during 20 December 2008 to 30 July 2009.

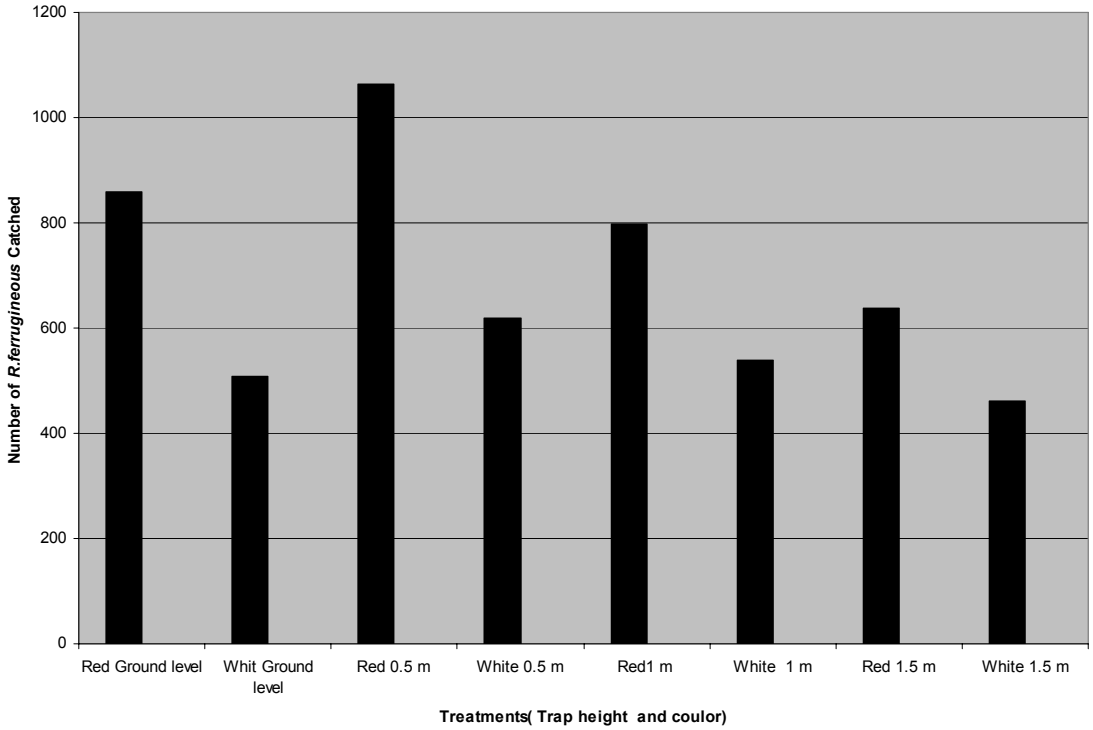

Fig. 6. Effect of *R. ferrugineus* Oliv. aggregation pheromone traps' height and color on the total number of caught weevils at Al-Rahba (Abu Dhabi) during 20 December 2008 to 30 July 2009.

The Infectivity of Entomolpathogenic Fungi *Beauvaria bassiana* and *Metarhizium anisopliae* to *Rhynchophorus ferrugineus* (Olivier) Stages under Laboratory Conditions

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Keywords: *Rhynchophorus ferrugineus*, *Beauvaria bassiana*, *Metarhizium anisopliae*, bioassay

Abstract

Two entomopathoginic fungi, *Beauvaria bassiana* and *Metarhizium anisopliae* were evaluated for their pathogencity against the larvae and adults of *Rhynchophorus ferrugineus* under laboratory conditions. Red palm weevil stages were collected from the infested palm trees, Al-Hasa Governorate, KSA, and reared on pieces of sugarcane. Three concentrations of each fungi spores were prepared, 1×10^7, 1×10^8 and 1×10^9 spores/ml. Adults of *R. ferrugineus* (male and female) and larvae were treated by dipping and injection bioassay techniques. The control treatment was treated with sterilized water. After treatments, insects were transferred to jars containing sugarcane. Insects were observed for three weeks and the percent of mortality recorded. The results showed variation in mortality for different stages. Mortality percentages were 40, 55, and 60 for male, female and larvae, respectively, at a concentration of 1×10^7 spore/ml of *B. bassiana* when insects were treated by dipping technique. Mortality reached 80, 85 and 85% for male, female and larvae, respectively when treated by injection at the same concentration. Mortality percentages were 35, 50, and 60 for male, female and larvae, respectively at a concentration of 1×10^7 spore/ml of *M. anisopliae* when insects were treated by dipping. The mortality reached 70, 60, and 70% for the previous stages when treated by injection at the same concentration after ten days of observation. The percent of mortality increased with increasing concentration of fungus spores. The percent of mortality reached 70 and 80 for female and male when contaminated males were transferred to jars containing untreated females after 17 days of transfer. The percent of mortality reached 10% in the control treatment during that period.

INTRODUCTION

Date palm tree, *Phoenix dactylifera* L. is an important tree for people in the Middle East. The red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Curculionidae*: *Coleoptera*), threatens the industry of dates (Murphy and Briscoe, 1999; Ajlan et al., 2000). In the mid 1980s *R. ferrugineus* was discovered attacking palms in the Arabian Peninsula (Gush, 1997; Abraham et al., 1998). The pest quickly spread all over the Middle East as a fatal economic pest to the date palm trees, which is now causing severe damage to date palms all over the Middle East. Effective methods for management of the red palm weevils have been difficult to develop. Current tactics employed try to manage the weevil based on the application of large quantities of synthetic insecticide that are applied as preventive or curative procedures (Abuzuhairah et al., 1996; Abdulsalam et al., 2001). All pest control methods that were tried, including dusting, fumigating, aerial spraying, soaking of plants with pesticides, and even injecting trees with all sorts of chemical pesticides could never slow the spread of this epidemic. Current research projects attempt to find a solution to save the remaining palms. The biological control

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seems to be the best solution especially when incorporated within an IPM program (Gerber and Giblin-Davis, 1990; Khan, 1993). Entomopathogenic fungi are candidate to control this pest. Zimmermen (1993), Jevanad and Kannan (1995), Schmitz et al. (1993), Prenerova (1995) and Vimala and Prassad (1996) reported about 750 entomoparasitic fungi. *Metarhizium anisopliae*, *Beauveria bassian*, *Verticilium lecanii*, *Nomuraea rileyi*, *Paecilomyces farinosus*, *Coelmomyces stegomyiae* and *Entomopthora* spp. are the most common species. Many entomoparasitic fungi exist in the soil and on trees. Siti et al. (1994) isolated *B. bassiana* from oil palm. Sewify (1997) isolated *M. anisopliae* and *B. basiana* from soil and evaluated their pathogenity against some economic insects. *M. anisopliae* was successful to control larvae and adults of *Oryctes rhinoceros* and red palm weevil (Sundara et al., 1983; Prior and Arura, 1985; Fernando et al., 1995). Biological control might be the most successful and most promising alternative to traditional pest control. Entomopathogenic fungi are inexpensive, non-hazardous, could be produced in large scales, easy for application and their existence in the environment could cause successful natural maintaining control and reducing the pest population and maintaining them below established economic thresholds. Success in applying the biological control is often dependent on a thorough understanding of the organism involved, both injurious and beneficial, and their interactions.

The aim of this work is to evaluate the pathogencity of two parasitic fungi *Beauveria bassiana* (Balsam) and *Metrhisium anisopliae* (Metsch) to larval and adult stages of red palm weevil under laboratory conditions.

MATERIALS AND METHODS

Insects

The main culture of *Rhynchophorus ferrugineus* was reared on fresh stems of sugar cane. The culture started with specimens collected from highly infested palm tree farms at Al-Hasa District, Eastern Province, Saudi Arabia. Restriction measures and care was performed during transportation and experimental work to prevent insects escaping. *R. ferrugineus* stages were kept in glass jars (500 cc) having circular holes in the lid for ventilation. Cocoon were kept separately until emergence. Newly emerging adults were sexed, then each couple of virgin females and males were kept separately in glass jars each containing one internode of sugar cane (longitudinally split) for oviposition. The culture was maintained at 25±1°C, 70-75% RH. The laid eggs were collected daily by cutting sugarcane into small pieces, peeling and shredding with the aid of a razor blade. Eggs were maintained under similar conditions in petri-dishes of 9-cm diameter with wet filter paper, until they hatched. Then they were provided with fresh sugar cane when transferring the larvae to glass jars. Different stages of *R. ferrugineus* (larvae, males and females) were used for bioassay tests.

Fungi

Two isolates of *Beauvaria bassiana* and *Metrarhizium anisopliae* were obtained from Egypt and the USA. The two isolates were injected in adults of red palm weevil to obtain a pure and highly pathogenic isolate using single spore technique.

Alive adults, first sterilized by 70% ethanol, were then washed with sterilized water, then injected with the spore suspension of each isolate and placed under laboratory conditions until they died. Died adults were taken and sterilized again and placed on potato-dextrose agar medium (PDA) until the mycelium of fungus came out from the cuticle of the insect. One spore from the growing fungus was taken and placed on PDA medium and conserved in an incubator for 7-10 days at 25°C until complete fungus growth of which spores are used to prepare the stock of spore suspension.

Preparation of Spore Suspension

Three concentrations of spores suspension (1×10^7, 1×10^8 and 1×10^9 spores/ml) were prepared in sterilized water from the stock of spore suspension and counted using an

heamocytometer slide.

Biological Activity Tests

1. Susceptibility Test. Laboratory trials were conducted to evaluate the efficiency of *Beauvaria bassiana* and *Metrarhizium anisopliae* against the different stages of *R. ferrugineus*. Fifteen larvae (15 days old) and/or adults (male and female) were dipped for one min in spores suspensions of each concentration of each fungus. Another fifteen larvae or adults were injected with 10 µl of each concentration of each fungus. Adults were injected in their thorax and between the segments in dorsal surface of larvae. Larvae or adults in the control treatments were soaked or injected by sterilized water. After treatments larvae and adults were transferred to fresh sugar cane pieces. Insect stages were examined after 10 days from treatments and the percentages of mortality were recorded. An insect was considered dead if it neither moved nor responded by reflex movement, when touched. Every concentration was replicated three times. LC95s were estimated according to Finney (1971).

2. Contamination of Male with *B. bassiana* Spores. Two males of red palm weevil were treated with a concentration of 1×10^9 spore/ml of *B. bassiana* by dipping technique and released in jars (500 cc) each containing 10 untreated females. The percentages of mortality of both males and females were observed along 17 days of release. Four replicates were used.

RESULTS

Biological Activity of *Beauvaria bassiana* and *Metarhizium anisopliae* to *R. ferrugineus* Stages

Data in Table 1 show variation in mortality between the different stages of red palm weevil (RPW) when treated by dipping technique by a concentration of 1×10^7 spores/ml of *B. bissiana*, whereas the mortality percentages were 40, 55, and 60% for male, female and larvae, respectively, after ten days from treatment. The percent of mortality increased when the previous stages were injected by the same concentration of fungus. The percent of mortality reached 80, 85, and 85% for male, female and larvae, respectively. In the case of *M. anisopliae* the same concentration (1×10^7 spores/ml) gave 35, 50, and 60% mortality when the RPW stages were treated by dipping. While it reached 70, 60 and 70% mortality of female, male and larvae, respectively when injected by the same concentration of fungus. Increasing the concentration of *B. bissiana* to (1×10^8) slightly increased the percent mortality of larvae and female (50 and 65%) when treated topically (dipping). At a concentration of 1×10^9 of *B. bissiana* the percent of mortality increased to 60, 70, and 85% when RPW stages were treated topically while it reached 95, 95, and 100% mortality of male, female and larvae, respectively, when injected. In case of *M. anisopliae* the same concentration (1×10^9 spores/ml) gave 50, 60, and 75% mortality when the RPW stages were treated by dipping while it reached 100, 90 and 90% mortality of male, female and larvae, respectively when injected.

Table 2 shows the lethal concentration (spores/ml) of both fungi to 95% of RPW treated stages. The data revealed that RPW larvae were more tolerant to *B. bissiana* compared to male or female when treated by dipping technique. Females were more susceptible to the used concentration of this fungus when treated by both applied techniques. The larval stage and males were more susceptible to *M. anisopliae* at the used concentration when treated by dipping. In general RPW treated stages were more affected by both fungi when injected than when dipped.

Contamination of Male with *B. bassiana* Spores

The efficiency of released contaminated male with *B. bassiana* at concentration of 1×10^9 spores/ml on the mortality of both males and females along 17 days from exposure were also studied using the dipping technique. Data in Figure 1 reveal that the percent mortality of both male and females increased with increasing the time of exposure. After

a day of exposure the percent of mortality reached 10 and 20 while it reached 70 and 80 for females and males after 17 days from exposure. The percent of mortality in the control treatment reached 10% after 17 days.

DISCUSSION

The results show that *B. bassiana* and *M. anisopliae* had a good effect on 15-days-old larvae, male and females of RPW at high concentrations 1×10^9 spores/ml whereas; more than 90% or complete mortality were achieved after 10 days from exposure mainly when the spores were injected inside the body of insect stages. This technique facilitates the germination of spores and fungal growth. In the meantime treated insects by dipping technique showed moderate toxicity to larval and adult stages either males or females after the previous period of exposure. Once again toxicity increased with exposure time and that might depend on the amount of germinated spores that are sufficient to suppress the target. However, the tested concentrations even the high ones did not show complete mortality (100%) to larval or adult stages of *R. ferrugineus* over the exposure periods when treated by dipping technique. These concentrations were considered very high compared with other insecticides that are recommended for red palm weevil's control.

The data refer that the released contaminated male could be used in the field to reduce the population density of RPW because it has the ability to fly a long distance to meet females and the spores of fungi are transferred to females during the intercourse. This technique might be useful to control RPW if the fungus is highly pathogenic, then when it reaches the insect cuticle the spores germinate and kill the insect.

CONCLUSIONS

In conclusion, *B. bassiana* and *M. anisopliae* are effective against larval and adult stages of *R. ferrugineus*, and might be suitable to incorporate in the control programs of *R. ferrugineus* as a protective or curative agent. The extra usefulness could be achieved if the formulation of those fungi is implemented in the integrated pest management programs (IPM) that are delivered to control the palm insect pests. The advantages of long lasting efficacy and mode of action are that they are different from organophosphates, carbamates and pyrethroids, in addition to being of low toxicity to mammals and the environment. Recent concern of Gulf countries about side effects of pesticides on the environment has resulted in the restriction in the use of many toxic products (Gush, 1997). Innovative biological control agents which are safer to beneficial and bee is recommended to be used for IPM programs (Zillekens, 2000). Lastly, current efforts are examining the potential developing of a biopesticide and focused on IPM, involving surveillance, pheromone lures, cultural control and chemical treatments for the management of *R. ferrugineus* (Moura et al., 1995; Abrahame et al., 1998; Ajlan et al., 2000; Abdulsalam et al., 2001; Shawir, 2006).

ACKNOWLEDGEMENTS

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Tables

Table 1. Mean Mortality percentage of Red palm weevil larvae and adults exposed to *B. bassiana and M. anisopliae* spores for 10 days.

Stage	Concentration (spore/ml)	Dipping *B. bassiana*	Dipping *M. anisopliae*	Injection *B. bassiana*	Injection *M. anisopliae*
Larva	1×10^{7}	40	35	80	70
	1×10^{8}	50	80	85	90
	1×10^{9}	60	50	95	100
Female	1×10^{7}	60	60	85	70
	1×10^{8}	65	40	70	80
	1×10^{9}	85	75	100	90
Male	1×10^{7}	55	50	85	60
	1×10^{8}	55	50	60	75
	1×10^{9}	70	60	95	90

Table 2. Lethal concentration of *B. bassiana* and *M . anisopliae* to 95% of red palm weevil stages after exposure for 10 days.

Stage	Dipping *B. bassiana* (spores/ml)	Dipping *M. anisopliae* (spores/ml)	Injection *B. bassiana* (spores/ml)	Injection *M. anisopliae* (spores/ml)
Larva	59.2×10^{8}	5.3×10^{8}	8.6×10^{8}	5.4×10^{8}
Male	31.2×10^{8}	11.8×10^{8}	9.9×10^{8}	11.8×10^{8}
Female	15.8×10^{8}	25.4×10^{8}	5.4×10^{8}	11.4×10^{8}

Figures

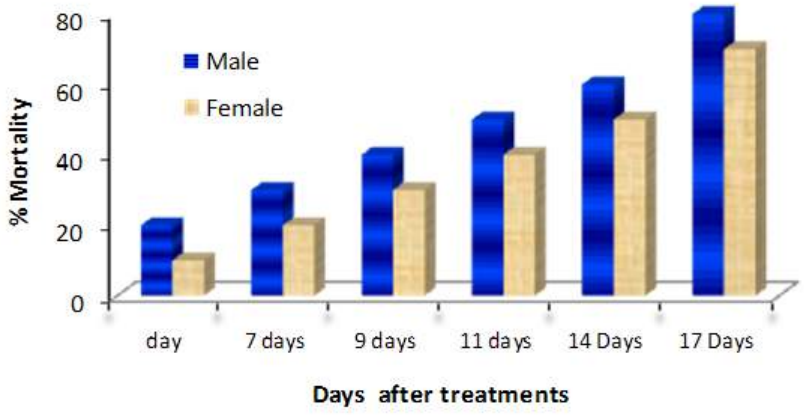

Fig. 1. Effect of contaminated released male with *B. bassiana* at a concentration of 1×10^{9} spores/ml on mortality of males and females of Red Palm Weevil.

Metwaly Endotherapic Injection Method for Palm Trees to Control the Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier)

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Abstract

The amended Italian Tree Vital Machine, tree vital endoplasm proved its efficiency as a very effective injection machine for palm trees, to control the red palm weevil (*Rhynchophorus ferrugineus*), in Italy, Saudi Arabia and Egypt. Such a patented machine has been used to inject 6 groups of 30 *R. ferrugineus*-infested palm trees (5 trees per group) by 6 insecticides (one/group). The insecticides were as follows: abamictin, oxydemethion, fipronil, imidacloprid, mitomyl and azdrachtin.

This method of application was found highly economical with less environmental hazards, and safe for the users.

Keywords: *Rhynchophorus ferrugineus*, insecticides, injection

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleopetra*: *Curculionidae*) is a very serious pest of cultivated palms in many countries in southern Asia, North Africa, Europe, the Gulf states, and the middle East. The weevil could invade many other areas and countries where date palms, coconut palms, sago palms, talipot palms, oil palms, royal palms, sugar palms, toddy palms, serdag palms, nibong palms, areca palms and some other ornamental palms are grown (van Derlaan, 1981).

Injection of insecticides, to control the palm tree pests, by pipes, proved not to be safe for the users, had many environmental hazards, were time-consuming and not economical. Therefore, the present work was conducted to evaluate one of the patented Italian tree vital machines as an endotherapic method for palm tree treatment.

MATERIALS AND METHODS

The application of the tested insecticides for the tested palm trees was carried out by adoption of the endotherapic method recommended by Metwaly (2008-2010) using a new model of his patented Tree Vital Machine for palm trees-injection. Five palm trees per tested insecticide, the tested insecticide concentrations ranging from 100 to 200 ml/L distilled water.

Injection probes were achieved by an electrical driller (1000 rpm). Palm trees of 35, 50 and 100 cm in diameter were infected. In order to evaluate the efficiency of both the tested insecticides and adopted injection new machine (Tree Vital Machine) the following parameters were investigated:

- Effect of the palm tree-diameter (35, 50 and 100 cm) on the time of injection.
- Effect of injection-machine pressure (1, 2, 3, 4, 5 and 6 bars).
- Effect of post-treatment period (3, 7 and 14 days, then 1, 2, 3 and 5 months).

All means were compared using the least significant difference test (LSD) at P=0.05. Also, the completely randomized design with 5 replicates (one palm tree = replicate) was used.

RESULTS AND DISCUSSION

The field observation on the insecticide-treated palm trees, by the endotherapic method using the patented Italian Tree Vital Machine to control the red palm weevil, *R. ferrugineus* (Olivier), revealed that all the subject insecticides were efficiently able to

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control the different stages of the red palm weevil inside the infested palm trees. On the other hand, the insecticide oxydemethion proved to be the most virulent one (nearly 98% mortality) as compared with the insecticide abamictin.

28 palm trees of 30 completely recovered from *R. ferrugineus* infestation (i.e., nearly 95% recovery). Dissection of some recovered palm trees showed that nearly none of the alive stages of the subject insect were present.

Observations on the effect of the palm tree diameter on the required time for trunk injection indicate that trees with 100 cm in diameter needed significantly less time (0.5 min) than those of 35 cm in diameter. Also, when the Tree Vital Injection Machine was adjusted at pressure of 5 bar the required injection time was very small (0.5 sec.) when compared to the corresponding values at 1, 2, 3 and 4 bars.

In addition, the present findings showed that both the adjusted pressure of the subject injection machine and the diameter of the treated palm tree had a significant effect on the required time for injecting the tested insecticides. The diameter of 50 cm and pressure of 5 bars recorded a considerable decrease in the time required for the insecticide injection by such machine, where only 0.24 s was needed as compared with the corresponding values of 35 cm and 1 bar, respectively.

Previous experiments on 600, 3500 and 5000 palm trees in Italy, Saudi Arabia, and Egypt, respectively, using the same Italian Tree Vital Machine to control the red palm weevil and other insect pests of palm trees, could confirm the present findings and many emphasize the promising role of a such endotherapic method for the palm tree by using the Tree Vital Machine.

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The Early Detection of Red Palm Weevil: a New Method

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Keywords: palm tree, red palm weevil, pest control, early detection, electronics in agriculture

Abstract

The red palm weevil (*Rhynchophorus ferrugineus*), or RPW, is considered one of the most dangerous pests facing palm trees in over 35 countries (including the GCC, Mediterranean, East Asia and part of Europe). Many attempts have been made to deal with this deadly pest, often referred to as the "palm cancer". As of yet, however, no viable method to detect the pest in its various forms has been developed. This document gives an overview of the current detection methods and introduces a totally new technique for the early detection of the RPW. The method is based on a combination of medical, computer, and electronic technologies. Because the larva is the most dangerous phase of the pest due to the direct damage it inflicts on the infected tree, this document concentrates on this phase of the life cycle. Several graphics and photos are exhibited showing results of the tests that were performed in the course of developing this method. The paper also suggests new quarantine protocols that could help improve the efficiency and effectiveness of post-infection strategies. And finally the paper suggests possible directions for future research and product development.

INTRODUCTION

Of all the pests and insects that attack palm trees, red palm weevil (*Rhynchophorus ferrugineus*) is arguably the most virulent. The most comprehensive RPW website[1] publishes quarterly data on the infestation rates of this deadly pest, and the numbers are growing exponentially. Affected countries (about 40 by now) include members of the GCC and others in the Mediterranean, East Asian and Southern European regions.

Finding an efficient solution to the RPW problem will not only save a "blessed" tree[2], but would also help farmers and governments reverse the mounting financial losses that have resulted. Although there are no specific studies on the economic impact of this problem, estimates place annual losses in multi-billion dollar ranges. The direct costs include the value of the destroyed trees and their potential date crop, the cost of trapping and other quarantine methods, and the huge budgets allocated to the various chemical treatments. The indirect costs are also substantial. The most significant of these is the restricted movement of trees, especially their offshoots. These restrictions result in drastic cuts in trading not only among countries but also between different regions of the same country.

The "palm cancer", as the RPW is often termed, has been the subject of intensive research in recent years. Most studies conclude that effective treatment is unlikely to be achieved in the absence of effective early detection. The term "early detection" essentially refers to the detection of infection while at the larva stage. The life cycle of the RPW starts with an adult female laying approximately two hundred eggs on new growth, either at the base of young leaves or in open lesions on the plant. Each egg hatches into a white legless larva. The larva will feed on the soft fibers and terminal buds, tunneling through the internal tissue of the tree, often excavating holes in the trunk. Scientists agree that it is this burrowing of the larva into the palm heart that causes the most mortality. By the time

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[2] Reference to the fact that palm trees are often mentioned in the Quran and the Bible.

the larva pupates, the damage that has already been inflicted is so serious that it eventually kills the host.

In addition to the long dormancy period, another problem complicating treatment is the rapid spread rate. Scientists believe that the main cause of the high spread rate is human intervention. The transportation of infested young or adult date palm trees and offshoots from infected to uninfected areas is the main culprit here.

Currently, there is a wide belief among policymakers, researchers, and farmers that early detection would help save thousands (if not millions) of healthy trees through the use of simple measures to quarantine the infected trees and protect the uninfected trees and offshoots. Potentially, then, early detection could help win the war against the RPW. In this short paper, we propose a test-proven early detection method that we believe could change the battle field.

The next section reviews some of the main methods currently in use in the war against the RPW pest and their limitations. Our proposed methodology will then be outlined along with a description of the initial testing performed. Conclusions and recommendations are presented in the last section.

OVERVIEW OF CURRENT METHODS

Over the years, the standard approach for dealing with the RPW problem has been to exterminate adult pests. This is usually done through chemical treatment (usually involving the injection of various insecticides inside the tree). There are two main problems with the standard approach. The first is that by the time the infection is detected and the treatment begins, the damage is so serious that the tree usually dies anyway. The second problem, also related to late detection, is the spread of the infection beyond the original area due to the transportation of infected trees and offshoots. This late detection of the presence of the weevil has long constituted a serious problem in the fight against the pest, and has led to a flurry of research aimed at early detection. Despite this, no safe techniques for early detection have been devised. A brief survey of the current detection methods follows.

A popular and well established technique entails the use of traps to determine the presence of RPW within certain perimeters. The major drawback with this method is that at most it only defines a general area where there is an infestation. In this way, it indicates the presence of the pest within that perimeter but could neither identify the specific tree or offshoot that is infected nor the extent of the damage that is already inflicted.

Another method involves the visual observation of infestation symptoms. This method relies on the human eye to detect the weevil. Clues such as chewed fiber rejects at the base of leaves or stems, folding and dying leaves, or smelly secretions are observed as possible clues to the presence of the pest. This method is seriously limited, however, because infested adult palms could live for years without displaying external symptoms.

A method that received substantial attention when first proposed a few years ago involves the injection of a virulent topical nematode worm into infected palm trunks. Once inside the weevil's body, the worm releases a lethal bacterium that causes death within three days. The strategy was only tested successfully in the laboratory, however. In real applications, the technique turned out to be deficient on one important account: the nematode attacks only mature weevils, not larvae. Since the weevils would have inflicted most of their damage by the time they are detected, the technique will not reduce mortality rates unless it is combined with an effective early detection method that allows farmers to inject the worm in trees that are identified to harbor the larvae.

The possibility of using audio detection systems (based on acoustics) has also been explored, but this method has only been tested in a laboratory setting and is still far from being implemented on a wide scale commercial basis.

Finally, and much more recently, there have been trials with trained sniffing dogs to smell the presence of larvae or mature weevils inside a tree truck. While this method may hold some future promise, especially, if the dogs could smell the larvae, mixed results have been reported. A question will probably remain as to how accurate and

reliable the results are, and where the exact location of the infestation is.

It is obvious from the above that much progress still needs to be made on early detection and diagnosis. The RPW spends most of its life cycle in a dormant larva state inside the tree trunk. By the time symptoms are manifested and treatment begins, it is already too late. Not only has that particular tree and its offshoots been already infested, but, since mature weevils travel fast from one tree to another, many of the surrounding trees within a particular perimeter would also be at risk. This leads to a much higher mortality than anticipated. That is why a lot of the contemporary research in this field is now focusing on issues related to detection, specifically early detection. As asserted by Faleiro (2006) in his authoritative review of 100 years of RPW management and treatment experience, the "early detection of Red Palm Weevil infestation in the field is vital for the success of any RPW-IPM programs". According to our own literature review as well as first-hand interviews, this sense of urgency is shared by most researchers and public policymakers in countries where RPW infestation is high.

PARAMETERS OF THE PROPOSED TECHNIQUE

The method proposed and explained in this paper focuses on diagnosis. Our goal is to develop a viable system to detect the RPW inside the tree in as many life cycle phases as possible. The method represents a totally new approach based on two simple hypotheses: a) an effective detection technology must provide definitive visual inspection of the pest, and b) detection must occur at the larva stage in order to achieve the desired mortality rate reduction. A technology like that would not only solve the major detection challenge of the weevil problem but would also eliminate most of the wasteful and costly guesswork that has characterized public policy in this domain.

Based on a thorough analysis of the current diagnostic routines, we decided to explore the usefulness of x-ray technology to this special detection problem. Despite the fact that current x-ray systems are mainly designed to identify human and animal tissues, this technology has been used in other applications, including a number of non-destructive tests and procedures. Additionally, there have been several attempts to use x-ray imaging to inspect wood surfaces for different purposes, but never before in pest control.

DETAILED SYSTEM DESCRIPTION AND TESTING

Practically palm trees can be divided into two main categories: a) offshoots and unplanted trees, and b) planted trees. Each category requires a different set of mechanical and functional requirements. The system we assembled was mostly designed for unplanted trees with plans in the works to deal with the planted trees in the field.

The System

A number of factors were involved in defining system hardware and software requirements, such as tree density (which could vary from one plant to another), pest tissue density, system mobility, etc. Once an optimum (representative) configuration was determined, we contracted with a specialized factory to build a customized x-ray system that included several components. System assembly and testing were performed in Italy.

The Testing Process

As already explained, and because the larva causes the most direct harm to the inner part of the tree, the priority was to detect the larva. Preparations for testing were performed in the following steps. Details about each step are also presented under each photo.

- First, we selected 4 offshoots (Fig. 3) with trunks 35-40 cm in diameter. These were x-rayed prior to any infection.
- Using a drill, we then created several tunnels with different sizes (8, 6, 4, 3 mm) (Fig. 5).
- Next, we inserted live larvae of different sizes into the trunks at different locations

(Fig. 6).
- Finally, we closed the holes with artificial jelly materials, and prepared the offshoots for another round of x-rays.

Test Results

Testing was performed using the specially designed radiography system and resulted in clear visual detection of the larvae in the radiographs. Specifically, the different tunnels made inside the tree trunk are clearly visible along with the larvae inside each tunnel (Fig. 7). The test showed the larva in different locations (Figs. 7-9). Furthermore, the movements of particular larvae can be traced by comparing different photo pairs (this is shown in Figs. 8 and 9). All the data were collected and stored in different formats which could easily be converted for further processing.

System Benefits

By performing tests such as the ones above at different local and regional testing stations in each country, infested offshoots could be identified and removed prior to shipping. It should be mentioned that the results that can be obtained in the field are unlikely to deviate much from those obtained in the lab. Confidence in the reported tests is therefore high.

The most important benefit of this system would be the resumption of offshoot trading among countries. This will allow farmers to recapture the income-earning potential of their offshoots. In recent months, this income had dwindled to negligible amounts in light of tighter regulations and stricter movement controls. The system would also allow the movement of larger unplanted palm trees of different sizes, such as those exported for their aesthetic and landscaping features.

In the future, it is expected that systematic detection would become part of all Integrated Pest Management (IPM) programs. The stationery scanning system which could be located at different borders, airports, seaports and inland locations can ultimately scan any unplanted tree regardless of its size.

Finally, the proposed system can also be used to help scientists better understand the biology of the RPW (the system could monitor its behavior day-by-day) and the extent of the damage it causes by monitoring its development closely inside the tree trunk.

Related System Functions

The purpose of this document is only to introduce this new method and prove its feasibility. However, it must be born in mind that a complete system would probably include a number of other features. For example, a conveyor belt to move the offshoots and trees being tested would be necessary, as well as one to sort the output between uninfected and infected trees. Components that perform the tracking, labeling, and tracing functions must also be considered.

CONCLUSIONS

The results obtained represent a breakthrough which could provide a new opportunity to combat a dangerous pest. The benefits of the system as a detection tool have been exposed. Further development needs to be performed to enhance the success rate of test results. One possible route for that is the use of advanced image enhancement and processing techniques.

It should be noted that this paper was not designed to explain all system features and functions but to report the successful results obtained and emphasize the importance of implementing this new method in the field in order to help eradicate this pest and ultimately consider it as a new detection standard as part of any effective IPM program.

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Figures

Fig. 1. Adult red palm weevil.

Fig. 2. Larva of the red palm weevil.

Fig. 3. Selected offshoot plants wrapped and transported to the test location: Italy.

a

b

Fig. 4. Plants were trimmed and removed from pots in preparation for the test.

Fig. 5. Drilling several holes in different sizes.

a

b

Fig. 6. Infestation with larvae (different sizes and location).

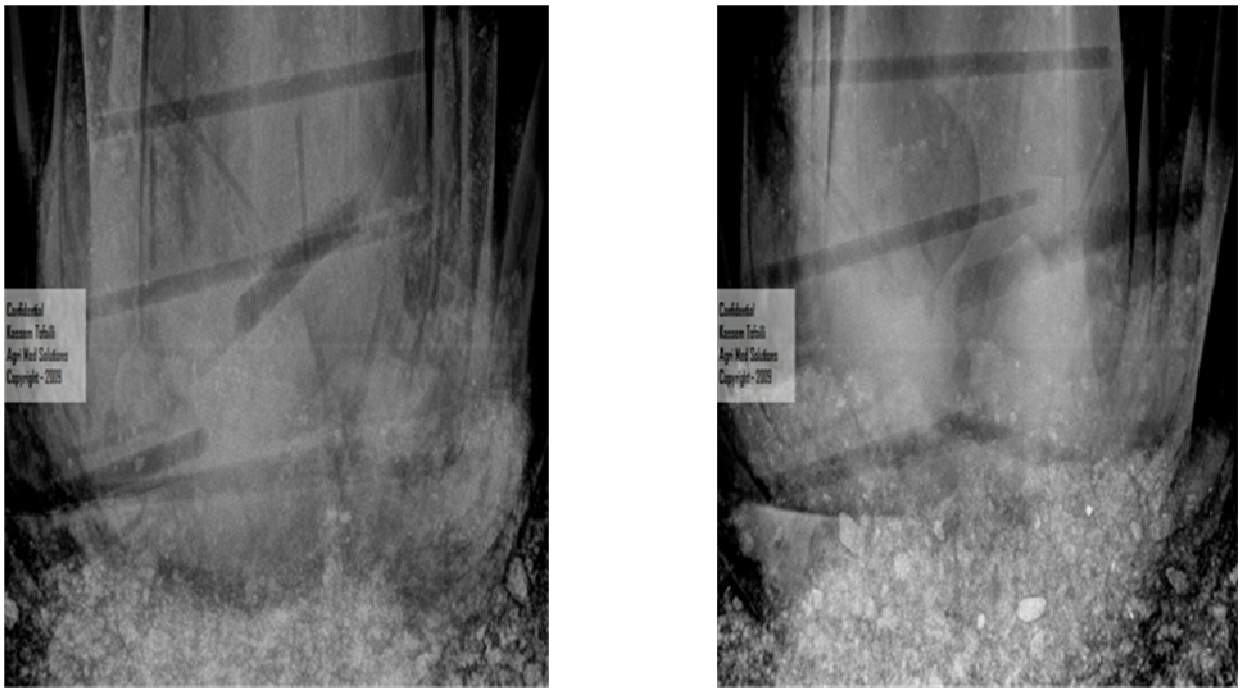

Fig. 7. Test showing all tunnels made using different size drills.

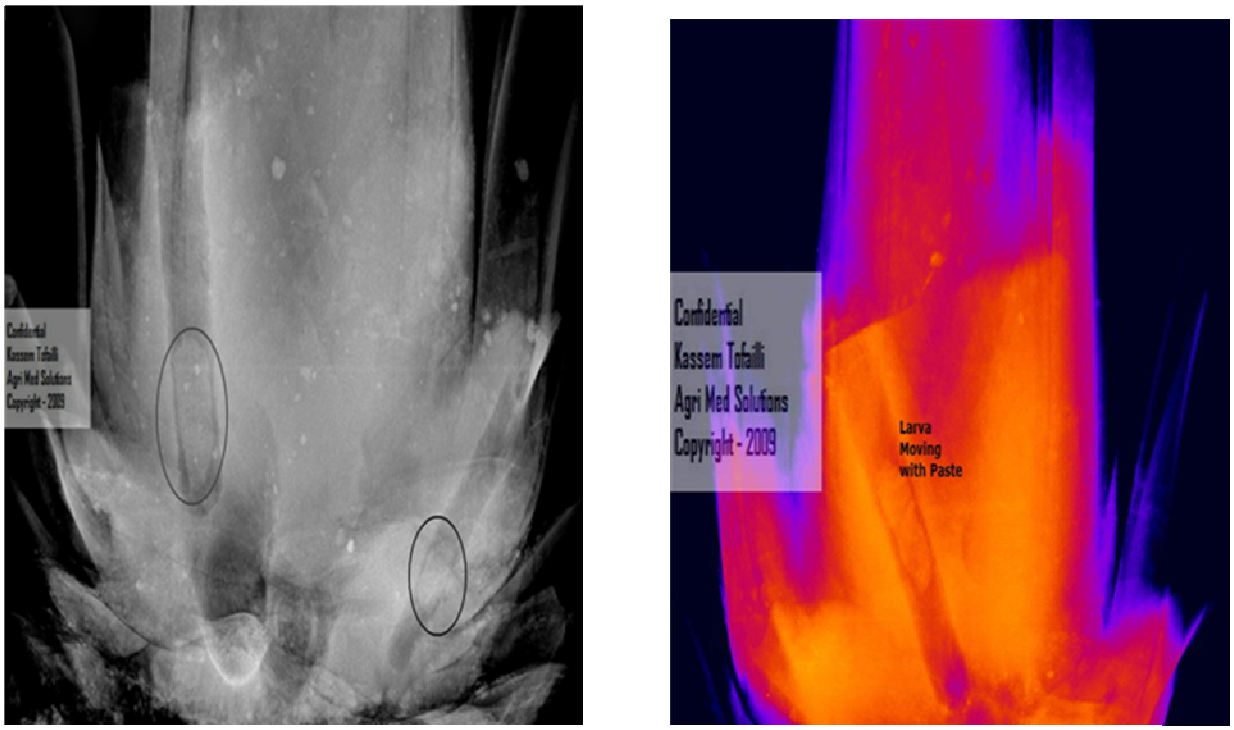

Fig. 8. Highlighted larvae inside the trunk.

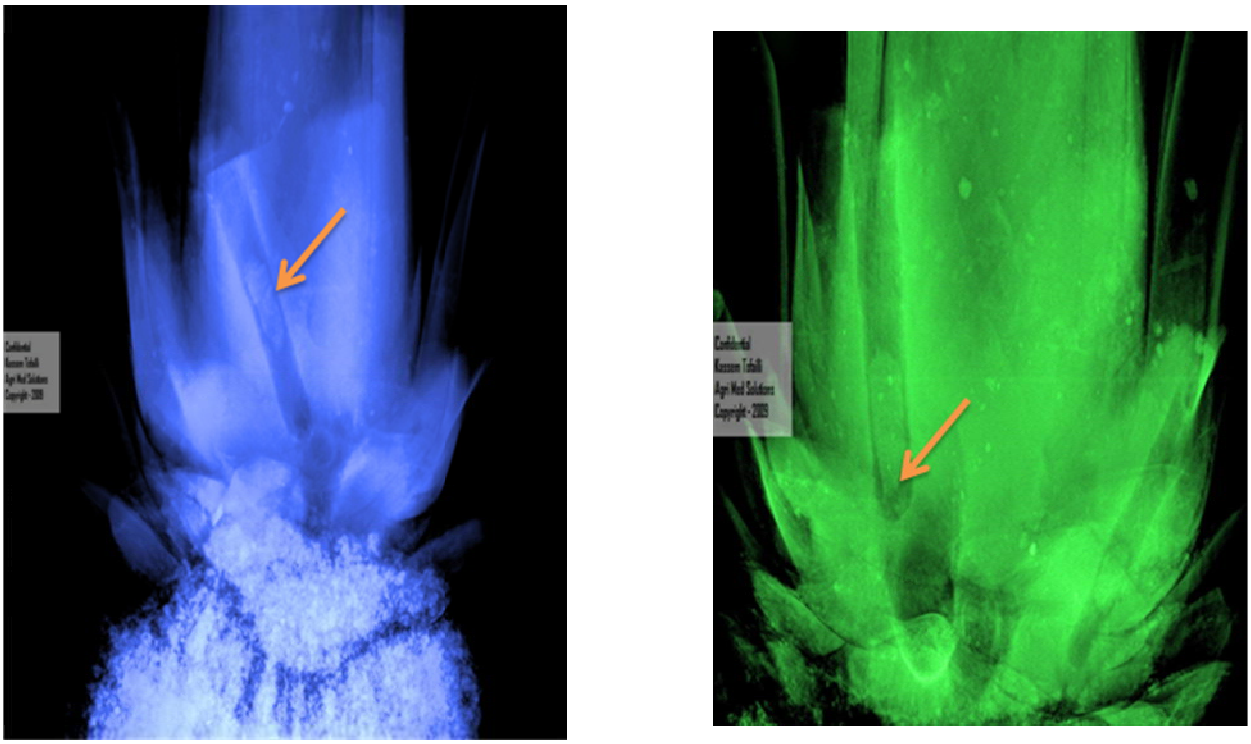

Fig. 9. Larva moving inside the trunk.

The Effect of the Egg Parasitoid *Pseudoligosita babylonica* on Dobas Infestation in Doaan, Shichawi and Asid (Hudramout, Yemen)

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Keywords: dobas bug, egg parasitoid, date palm, Hudramout, Yemen

Abstract

The best and most efficient method of biological control is the use of egg parasitoids, and most species used were from the family *Trichogrammatidae*, and the name of the dobas egg parasitoid (DEP) in Yemen is [(*Pseudoligosita babylonica* n. sp. (*Hymenoptera*: *Trichogrammatidae*)]. We have followed the existence and the impact of this parasitoid in Hadramout since 2005, we recognized it in 2007 in Gail Al-Halikah, it was identified in France (CIRAD) at the end of 2008 and it turned out to be the same type found in Iraq, which was found also in 2005, during 2008 and 2009. In the four generations of the dobas insect we followed the spread in Douan, Asid and Shichawi. We found that it exists in all villages of these regions. The proportion of parasitism differs from one area to another, significantly at the level of 5% and the percentage of parasitism declined from 23.6% in January 2008 to 2.53% in August 2009 in all the 13 villages in Douan. The heavy rain in October 2008 influenced the parasitism because it washed the honey dew from the leafs which is the mean food of the parasitoid, and in the district of Ridh Gosaiar where there was a clear significant difference between Wadi Asid and Chkawi; it was only 6% in Asid and 17% in Chkawi, which led to its effect on the severity of the injury, accordingly it was in Chkawi 6.5 eggs/leaflet, while it reached to 9.3 in Asid which confirms the clear relationship and positive impact in reducing the injury.

The adults of the DEP were present all the time of the dobas egg periods and approximately it moves from the old egg masses to the new for the second generation. It was also noted that the parasitoid was present in all plant heights of different palm trees up to more than 15 meter.

INTRODUCTION

Of the best and most efficient biological control methods is to use egg parasitoids, and most parasite species are of the family *Trichogrammatidae* which parasitize species of the *Lepidoptera* family, and are used globally and commercially, and it is a pity to say that all States with a significant dobas damage to date palm have not used any type of parasites or predator in their control programs. So far, a parasitoid has been recorded on eggs in the Sultanate of Oman, an *Oligosita* sp., Family: *Trichogrammatidae*, order *Hymenoptera* in the village Samail. Al-Katari (2006) found that the percentage of eggs that the parasite came out of reached between 6 and 25% in March and May 2005, respectively, and in 2008 he noted the spread of the parasite attributed from one location to another, and it did not exist in Kasip district, while the percentage of parasite eggs reached 60% in the village of Tiwi in eastern region and it is very clear that the parasitoid played a good role to control the date palm dobas insect (DPDI) .

In Iraq, it was noted that it reduces the population density of dobas eggs well. Hassan et al. (2007) also studied the life cycle of the parasite in the laboratory and found that the age of the parasite is 31 days at a temperature of 28°C, and pointed out that females and males of the parasite feed on the honey material produced by the dobas insect. It has been shown that when there is no honey the age of gender lowers. The parasites have been classified in Italy by Viggiani and Hasan et al. (2003) found a new type and named it [*Pseudoligosita babylonica* n. sp. (*Hymenoptera*:

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Trichogrammatidae)]. We have followed the presence of this parasite in Hadramout since 2005 before we recognized it in 2007 in Gail-Alhalikah village, and for identification we sent the samples to France (CIRAD) and found the same type which was found in Iraq.

This work aims to study the spread of the dobas egg parasitoid (DEP) and its relationship with DPDI in the most important areas and districts of coastal Hadramout (e.g., Wadi Duan, Asid and Chkawi) to give some observations about the behavior and the presence of DEP.

MATERIALS AND METHODS

During field visits to the regions, four samples from each site were taken. Each sample was the terminal meter of a leaf from the fourth or third round in one tree. Trees were selected at random and after confirming the presence of eggs to the same generation, which will hatch later within one month. The leaflets were cut out by scissors and the rest were cut into five pieces, placed inside a small plastic bag in preparation for transfer to the laboratory and examined under a binocular. The following information was calculated: (1) the number of cluster eggs between all the leaflets of the sample and the number of eggs per leaflet was measured (using the equation improved by Hubaishan, 2007). The number of egg clusters divided by 3.2 = no. of eggs per leaflet, (2) the percentage of parasite eggs (PPE) in five clusters.

In Duan district 13 locations (villages) are representative for the whole valley, namely, (Al-Ribat, Al-Gwairah, Rehab, HisunAlgopob, Lagrat, Bidah, Bilad Almah, Kailah, Al Gahee, Al-Arsimah, Saeef, Gar-Laswad, Al-Gizah) and in all of those areas date palm trees are planted under the spate irrigation system, and approximately 97% of all the trees were of the 'Gazaz' variety.

In the district of Al-Raidah and Gosaiar there are four valleys famous for date palm plantation and the concatenation from east to west is as follows: Chkawy, Bidish, Assid and Ragdoon, all were infested by DI and we included only Chkaoy and Asd under this research study. The date palm trees were irrigated by the sustained system from Al-Ayoon, and about 95% of the trees are of the 'Sogotrai' variety. The villages in Asid were Al-Milahees, Sfal-Hibud, Hubid, TahitTareeg and Dibag, in the valley of Chkawy they were Algeel, Dicoman, Al-Gobairah, Al-Gerhabah and Al-Atim.

The data were analyzed by using the Genestat 5 program for statistical analysis.

RESULTS AND DISCUSSION

The First Registration of Dubas Egg Parasite (DEP) in Yemen

Through our follow-up we observed the emergence holes in the eggs where the DEP comes out since 2005, and we call them damaged eggs and in 2007 we saw the parasite coming out from these. Therefore, samples were collected and send to Dr. Laurence Ollivier in CIRAD/France for identification. They were categorized and found as being from the family *Trichogrammatidae*, order *Hymenoptera*, and the scientific name *Pseudoligosita babylonica* n. sp., and thus we registered this parasite for the first time in Yemen. It was the same parasite on Dopas eggs as in Iraq which had been labeled there in 2003, as a new parasite by the Italian G. Viggiani (Hassan et al., 2003).

Spread of DEP and Its Relationship to DI Injury

1 Duoan District. The results in Table 1 show that the dobas egg parasitoid (DEP) exists in all villages of Wadi Duan. The percent of parasitism differs from one area to another significantly at 5% and ranging from 1% in the Gar-Laswad to 41% in Rehab over the two generations, autumn 2008 and spring 2009. In view of the change in the two generations, it was noticed that there was a non-significant decrease in the percentage of parasitism, from 18.3 to 13.2% in generations autumn and spring, respectively. This may be because the presence of the parasite was very much affected by the heavy rainfall in October 2008, which washed the honey material from the leaves, which the parasitoid feed on, particularly in the regions Al-Jahee, Al-Arsimah, Saeef, Gar-Laswad and Al-

Gizah. This relation was proved also by Hassan et al. (2004) (Table 1).

2. Asid and Chkawi Vallevs in Al-Raidah and Gosaiar District. The results in Table 2 show that there are significant differences between villages in PPE and also in the severitv of the infection, which means each region has its particular circumstances. But it is verv clear between the vallevs where it was 17% in Chkawi, while in Asid onlv 6.2%. It is noted that the differences in the PPE between the generations were significant, but not accompanied bv a significant difference in the DI, suggesting that there were some factors influencing the PPE and lower it while the DI remained similar and this result is identical with Wadi Doan and it can also be argued that torrential rains that fell on all those areas at the end of October 2008 led to the low rate of parasitism in the next generation (Table 2).

The Behavior of the Parasite and Time of Existence

Notes from the following chart show that the egg parasitoid is available to malfunction eggs and continues to develop its eggs inside the DE, from the beginning of egg laying starting from November 15 to the first week of March to the end of the egg laying period of the spring generation and then the DEP cannot be seen only after the new eggs of the next generation start from mid-April to mid-September, and its presence increases with the existence of artificial honev, on which the adults of the parasitoid feed.

The question remains where those small insects go. Are thev going to another host in a different crop? Or do thev overwinter in the dobas eggs which did not hatch, sometimes reaching 40%. After further investigation we found that the DEP exist all the time and it does not overwinter in all areas of our study. These results were totally different from those in Iraq according to Hassan (2007) where he said that the DEP has only two generations, similar to the dobas insect. At the site of Asid we had the opportunity to identifv the presence of the DEP at three heights of palm trees (15, 9, 2 m) and initial results indicate that the DEP was presence in all of those levels where the percentage of parasite eggs were 7.8, 11.4, 1.6, respectively with an infestation of dobas 9.1, 15.3 and 8.1 eggs/leaflet, respectively.

CONCLUSIONS

1. Dobas egg parasitoid (DEP) *Pseudoligosita babylonica* has a big role in reducing the damage of the date palm dobas insect .
2. The existent of DEP was different from one valley to another and from one village to another.
3. The parasitoid was present in each height of the palm trees, from the offshoots to more than 15 m height.

RECOMMENDATIONS

1. The relation between the DEP and the infestation of dobas should be investigated prior to anv planning for chemical control application, so as to give the nature of biological control a good chance to control the infestation of dobas.
2. Study the behavior of the parasitoid in nature (its hosts of other different crops).
3. Study the possibilitv of rearing the DEP on another hosts which is easilv reared in the laboratory (such as the grain butterfly or flour butterfly, etc.).

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Tables

Table1. The relationship between the percentage of parasitism and the severity of injury in 13 villages of wadi Dooan.

No	Villages	% of dobas egg parasitoid DEP					Dobas infestation (egg/leaflet)				
		Jan 2008	Aug 2008	Jan 2009	Aug 2009	Mean	Jan 2008	Aug 2008	Jan 2009	Aug 2009	Mean
1	Al-Ribat	16.5	5.9	8.8	6.5	9.4	4.7	8.98	7.15	13.5	8.58
2	Al-Gwairah	41.1	18.5	2.9	1.5	16.0	3.8	3.12	4.15	6.7	4.44
3	Rehab	34.1	21.0	61.7	4.75	30.4	5.54	2.92	8.27	9.73	6.62
4	Algopob	3.2	28.0	39.7	1.75	18.2	12.65	4.22	5.23	9.25	7.84
5	Lagrat	20.5	45.7	0.7	3.05	17.5	3.8	1.47	2.73	7.75	3.94
6	Bidah	6.7	15.8	10.8	4.17	9.4	11.45	5.47	8.13	7.75	8.20
7	Bilad Almah	37.8	7.2	14.0	1.27	15.1	8.74	3.42	3.68	5.45	5.32
8	Kailah	8.7	16.8	22.3	3.22	12.8	2.75	3.81	1.72	3.6	2.97
9	Al.jahee	33.8	48.1	0.0	1.85	20.9	2.94	2.25	2.03	5.45	3.17
10	Al-Arsimah	21.5	12.5	4.3	3.75	10.5	3.68	5.00	6.65	6.25	5.40
11	Saeef	27.3	1.4	0.8	0	7.4	13.83	7.63	3.92	13.2	9.64
12	Gar-Laswad	39.4	2	0.0	0	10.3	7.25	4.30	8.15	13.7	8.36
13	Al-Gizah	0.7	10.1	5.7	0	4.1	5.38	2.16	2.48	5.35	3.84
Mean		22.4	17.6	13.2	2.45	14.0	6.65	4.21	4.94	8.28	6.02
LSD		17.33	27.55	12.25	4.16	20.02	5.286	3.281	4.254	6.227	3.281
CV%		21.2	60.7	14.8	46.2	61.2	15.3	21.3	14.2	11.7	30.2
LSD between generations		11.11 and the CV 48.9%					1.82 and the CV 38.2%				

Table 2. The relationship between the percentage of parasitism and the severity of injury in 10 villages of Wadi Asid and Shkawee.

No	Villages	% of dobas egg parasitoid DEP					Dobas infestation (egg/leaflet)				
		Jan 2008	Aug 2008	Jan 2009	Aug 2009	Mean	Jan 2008	Aug 2008	Jan 2009	Aug 2009	Mean
1	Al-Milahees	23.7	2.2	2.7	16.4	7.1	4.4	10.1	14.2	3.95	9.41
2	Sfal-Hibud	9	7.7	4.6	46.7	19.7	2.5	5.92	6.22	8.10	6.75
3	Hubid		13.3	2.1	18.8	11.4		13.3	11.8	8.77	11.29
4	TahitTareeg	5.5	0.8	4.9	33.5	13.1	4.03	5.72	10.6	5.75	7.37
5	Dibag	38.7	11.8	11.5	62.2	28.5	5.1	7.12	9.8	7.50	8.16
6	Algeel		18.6	3.8	40.0	20.8		7.10	5.1	6.40	6.19
7	Dicoman		17.6	10.9	42.0	23.5		6.00	1.6	1.65	3.10
8	Al-Gobairah		39.9	8.5	54.7	34.4		14.5	6.5	5.07	8.69
9	Al-Gerhabah		28.3	4.5	30.3	21.0		11.5	5	2.77	6.41
10	Al-Atim		32.2	6.4	56.8	31.8		6.00	1.3	1.42	2.91
Mean		19.2	17.2	6.0	40.1	21.1	4.0	8.73	7.2	5.14	7.03
LSD			14.54		24.68	16.43		6.064		6.762	4.857
CV%			% 5.9		9.7	45.3		% 11.3		28.4	25.6

Villages from 1-5 in Wadi Asid and from 6-10 in Wadi Shikawee.

Figures

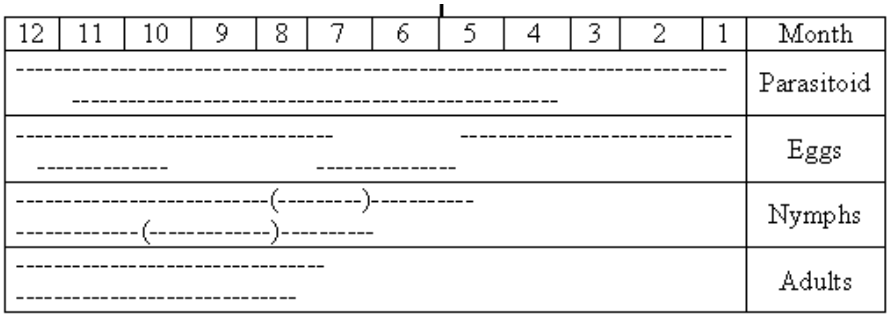

Fig 1. The periodical existence of dobas insect stages and its egg parasitoid in coastal Hudramout.

Economic Impacts of Date Palm Infestation by the Green Pit Scale Insect (*Palmapsis phoenicis* Ramachandra Rao) in Northern Sudan

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Keywords: date palm, green scale insect, northern Sudan, economic impacts

Abstract

A wide area study to explore the cause-effect relationships between different factors affecting infestation by the green pit scale insect (*Palmapsis phoenicis* Ramachandra Rao) in northern Sudan and its main economic impacts was carried out in 2008. Primary data were collected from farmers using questionnaires and subjected to analysis by appropriate analysis techniques using SPSS. Main results revealed that the level of infestation of date palm trees with the green pit scale insect was not significantly related to soil type, intercropping, method of irrigation (flooding or drip irrigation) or irrigation interval whereas significant relationships were found between the level of infestation and use of permanent irrigation, regular fertilization and farm size.

There are also no significant differences in the total cost of production between the upper, middle terrace soils and islands and the TC varies even among irrigated date palm trees depending on the source of irrigation (from the Nile or underground water). The total cost of production using groundwater is higher by 63% than irrigation from the river Nile. Differences in farmers' education, age, and infestation of date palm trees by other pests do not significantly affect the total cost of production

The proportion of farmers who produce less than half a sack/tree/year (about 50 kg per tree) is 68% of the total farmers in the studied area as a result of infestation by the green scale insect and weak palm care. Total cost of production for farmers who produce more than half a sack/tree is higher by 53% than that of farmers who produce about half a sack without a significant difference between the two groups. Significant differences in the total cost of production at 10% confidence level between different age categories is evident in the analysis. Low date fruit prices and poor marketing as a result of infestation represent the most important market level impacts of the increasing infestation by the green pit scale insect in the area.

INTRODUCTION

Statement of the Problem

The date palm industry in Sudan is facing many serious problems which are low yields, lack of appropriate packing and presentation as well as limited processing of date products. The estimated average yield of a date palm tree in the main date growing areas in Sudan is around 20 kg, which is very low compared with an average yield of more than 100 kg in other date growing areas in the region (FAO, 2002). As a result of the high cost of production and low prices of the produce, farmers tend to pay less attention to date palm trees.

In Sudan, the green date palm pit scale insect (*Asterolecanium phoenicis* Rao.) is considered the key pest. This genus, a native of central Asia (Iran), (Ezz, 1973) was not known in Sudan before 1989 when it was firstly reported by Ali (1989) in the El Golid area, as a result of an introduction of some offshoots from Saudi Arabia in 1974. Later, the pest crossed the natural barrier of the Baja desert to invade Elgaba scheme (150 km south of Dongola, 400 km north of Khartoum) and has become a real threat to date palm

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cultivation in Northern Sudan. The infested area in El Golid, Elgaba and Old Dongola is about 5000 ha, extending over 60 and 50 km along the west and east banks of the river Nile respectively. The newly reported infestation in Artigasha Island, Burgaig scheme and Orbi in Dongola area, Abuhamad in the River Nile State (23000 infested palm trees) and Khartoum State, provides evidence that the pest may continue to spread.

Estimated losses in production due to infestation range from 30-50 to 5 kg per tree (Ali and ElNasr, 1992). The losses may range between 85 and 90% according to infestation rate, variety infested and management conditions (Ahmed, 2001, 2004). In the past, and due to lack of indigenous knowledge of the nature of this pest, control efforts were not successful; hence the level of infestation steadily increased.

Objective of the Study

The objective of this study was to determine the economic impacts of infestation by the green pit scale insect and to evaluate knowledge and practices of farmers in dealing with it.

METHODOLOGY

Primary data were used in this study and collected directly from date palm growers in northern Sudan using a specially designed questionnaire. Data were collected through a field survey in 2007 and 2008 from 171 farmers in the infected areas. A stratified random sample according to the soil type (alluvial or high terrace soils) was implemented and data were analysed using the statistical analysis software SPSS. A secondary data source was also used to draw out results and conclusions.

RESULTS AND DISCUSSION

Distribution of Infestation and Testing Relationships

Results showed that most palm trees with a high degree of infestation were in Elghaba area (53%) which also contains 30% of the total infestation of the green pit scale insect in the state (Table 1). There are no significant differences in incidence among different regions surveyed, but there is a significant difference in the degree and seriousness of the infestation in Alghaba, Algolid and Old Dongola which shows the similarity of those areas.

The proportion of palm irrigated regularly is 80% of the total palm in all regions of the study (Table 2), concentrated mostly in the Alburgaig area (30%) and Elghaba (27%). There is no significant statistical relationship between infestation and the permanent irrigation indicating a large disparity between regions in palm irrigation although it is known that the primary cause for the entry and spread of this insect is the lack of adequate attention, especially non-regular irrigation. It is found that 31% of the palm trees in the study area are irrigated by underground water and 22% from the Nile whereas 36% of them are irrigated from both sources. The significant relationship between source of irrigation and the incidence of infestation is an important signal as it was found that 39% of the infected palm trees are irrigated from both sources and the figure is 28% in the case of irrigation by wells and 22% of trees irrigated from the Nile. This relationship needs to be examined to clearly determine the proposed effect of irrigation water source on the incidence of infestation by the green scale insect. Table 3 shows frequency distribution of the source of irrigation in the southern part of the state.

There is significant relationship at 10% confidence level between soil types in all areas of study which shows similarity of land properties in the study area where palm cultivation and production is concentrated in the middle and upper terrace soils (68%) whereas 32% of the total palm trees are found in the upper terraces. However, there is no relationship between the degree of infestation and soil type of where the insect affects palm trees in the lower, middle and upper terrace soils similarly.

Farmers in Alghaba, Algolid and Dongola practise intercropping similarly (68% of farmers) whereas it is concentrated in Alghaba area (63% of the farmers who use

intercropping in the study area are found in this area). The low value of the chi-square (0.006) suggests similarity between these areas in intercropping (Table 4), but there is no statistical relationship between the level of infestation and intercropping.

There is a close relationship between the level of infestation and the date of onset of illness in the fields of farmers for the first time. Most high incidence (65%) is concentrated among palm trees which showed first symptoms of infestation between the end of the seventies and beginning of nineties of the last century, while the pest incidence of varying degrees was 15% of the total in the year 2000.

There is also a significant relationship between the level of infestation and the number of palm trees per farm. It is found that 58% of the high incidence of infestation is among farmers who have fewer than 100 trees. The rate is also high (44%) among older farmers in areas of Alghaba Algolid and Old Dongola. This can show difficulties faced by farmers in dealing with the pest due to high cost and effort required to deal with large numbers of date palm trees which is beyond their financial capabilities.

There is no significant relationship between the level of infestation and age of the tree where the pest affects all ages equally. Most infestation is among palm trees in the 10-20 year age (69% of the total incidence). Effects of infestation on productivity in ANOVA are shown in Tables 7 and 8.

This is consistent with the actual number of palm trees in this age in the study area with an average ratio ranging between 10-20 years (68% of the total palm trees) with no differences among regions regarding the average age of date palm trees.

The Impact of Infestation on the Cost of Production, Productivity and Marketing

There is a significant difference in production costs between the different levels of infestation with an average of 59 SDG per year. Tables 5 and 6 show the effect of infestation on the TC and its ANOVA. The cost of production reaches the lowest level at the high pest infestation and this could be due to the fact that farmers tend to reduce expenditure as infestation becomes more serious that can affect productivity and costs greatly.

Comparing the infestation this study shows that farmers in the Alghaba area expend the least money on date palm trees and this is consistent with the concentration of a large proportion of infected trees in this region and the reluctance of farmers to spend on palm trees that are seriously infected. The average cost in the southern regions is 66 SDG per year without significant differences among regions.

There is also no significant difference in production costs between the upper, middle terrace and islands despite the fact that production costs in the upper terrace soils is higher by 33% than in lower lands but this difference statistically non-significant. The effect of different soil types and their significance are shown in Tables 9 and 10.

Irrigation cost is the highest cost item among production costs. This is evident by comparing the total cost to farmers who irrigate palm trees on a regular basis with higher production costs compared with farmers who do not irrigate regularly by 127% with no statistical difference between the two groups. The effect of permanent irrigation and its significance is shown in Tables 11 and 12.

Production costs vary even among irrigated palm trees according to the source of irrigation where cost of production using groundwater wells is higher by 63% than using Nile water. This can be attributed to differences in the cost of irrigation through pumping groundwater compared to the Nile water. The effect of the source of irrigation and its significance is shown in Tables 13 and 14.

Tables 15 and 16 show that the level of education of farmers does not affect production costs without a significant difference on average between some levels.

There is also a difference in the total cost of production between farmers who work in professions other than agriculture and full-time farmers. There is a similarity in the average practices of the two groups as shown in Tables 17 an 18.

Further results show that there is no difference in production costs in the case of infestation by other insects, where the two groups did not differ significantly despite the

rise in cost by 11% in the absence of infestation by other insects. This can be explained by the weak attention by the farmers who suffered from other pests which might lead to reduction of spending on these farms.

The proportion of farmers who produce less than half a sack/tree/year (about 50 kg) is 68% of the total farmers in the study area due to the infestation by the green scale insect and weak palm care. Production costs for farmers who produce more than half a sack/tree is higher by 53% than that of farmers who produce about half a sack without significant differences between the two groups. These results are shown in Tables 19 and 20.

Significant differences in production costs at 10% level between different categories of age, show that the cost of palm production is highest for small palm trees (less than 10 years) as a result of the care given by the owners, contrary to the palm above 20 years of age. This conclusion is consistent with the concentration of infestation within the age group of 10-20 years (63%) in the southern areas so farmers become reluctant to bear any additional costs if the age exceeds 20 years with high infestation that significantly lowers dates productivity.

It is found that 61% of farmers in all regions of the study periodically clean palm to combat the pest, while pesticides are used by 13% of them.

Low date fruit prices and stagnant market due to infestation represent the most important consequences of the increasing danger of exposure to green pit scale insect. More than 70% of farmers said that the infestation, low fruit quality crops had led to a slump. Farmers' responses varied depending on the awareness about the seriousness of the insect.

CONCLUSIONS

Infestation of date palm with the green pit scale insect in northern Sudan is related to poor management of the trees. The significant relationship between the level of infestation and the use of permanent irrigation, regular and adequate fertilization and farm size is an important sign of the way infestation has spread in the area. Non significant relationship between the level of infestation and soil types, method and interval of irrigation also explain the invasion of new areas by the insect irrespective of soil type and irrigation regime. Cost of dates production is higher in the infested areas compared with the other pest free areas for all soil types, source of irrigation farmers age and education level although in some cases lower cost of production was found as a result of skipping some cost items. Low yield is evident in 68% of date palm trees in the studied area as a result of the infestation which is also associated with a 53% rise in the total cost of dates production. Low prices in the local market and reluctance of date traders from buying infested poor quality output is the most important market level impact that leads to low returns to farmers.

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Tables

Table 1. Distribution and significance of the level of infestation in the study area.

Location		Level of infestation			Total
		Low	Medium	High	
Algolid	Count	3	5	10	18
	% within location	17%	28%	55%	100%
Old Dongola	Count	0	11	12	23
	% within location	0%	47%	52%	100%
Alghaba	Count	3	20	25	48
	% within location	6%	42%	52%	100%
Total	Count	6	36	47	89
	% within location	7%	40%	53%	100%

Chi square = 0.261.

Table 2. Distribution and significance of permanent irrigation in the study area.

Location		Permanent irrigation		Total
		Yes	No	
Artigasha	Count	25	2	27
	% within location	93%	7%	100%
Alburgaig	Count	42	2	44
	% within location	95%	5%	100%
Algolid	Count	18	7	25
	% within location	72%	28%	100%
Old Dongola	Count	14	11	25
	% within location	56%	44%	100%
Alghaba	Count	38	12	50
	% within location	76%	24%	100%
Total	Count	137	34	171
	% within location	80%	20%	100%

Chi square = 0.216.

Table 3. Distribution and significance of the source of Irrigation in the southern areas.

Location		Source of irrigation		Total
		Nile	Wells	
Algolid	Count	13	7	20
	% within location	65%	35%	100%
Old Dongola	Count	10	7	17
	% within location	59%	41%	100%
Alghaba	Count	27	20	47
	% within location	57%	43%	100%
Total	Count	50	34	84
	% within location	60%	40%	100%

Chi square = 0.85.

Table 4. Distribution and significance of intercropping in the study area.

Location		Intercropping		Total
		Yes	No	
Algolid	Count	14	9	23
	% within location	61%	39%	100%
Old Dongola	Count	10	12	22
	% within location	45%	55%	100%
Alghaba	Count	41	9	50
	% within location	82%	18%	100%
Total	Count	65	30	95
	% within location	68%	32%	100%

Chi square = 0.006.

Table 5. Effect of infestation with the green scale insect on the cost of production.

Soil type	Cost (SDG/tree)
Non-infected	22.1
Infected	65.9

Table 6. ANOVA and significance statistics of the total cost of production between infected and non-infected trees with the green scale insect.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	1E+008	1	124834168	0.604	0.43
Within groups	2E+010	97	206690235		
Total	2E+010	98			

Table 7. Effect of infestation with the green scale insect on the cost of production by location.

Location	Cost (SDG/tree)
Algolid	78
Old Dongola	95
Alghaba	42
Total	66

Table 8. ANOVA and significance statistics of the total cost of production according to infection.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	5E+008	2	243208627	1.108	0.3
Within groups	2E+010	89	219564411		
Total	2E+010	91			

Table 9. Effect of soil type on the cost of production of date fruits in the northern state.

Soil type	Cost (SDG/tree)
First class	60
Upper terrace	81
Total	67

Table 10. ANOVA and significance statistics of the total cost of production in different soils.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	8E+007	1	80654322	0.353	0.554
Within groups	2E+010	87	228779676		
Total	2E+010	88			

Table 11. Effect of permanent irrigation on the cost of production of dates.

Permanent irrigation	Cost (SDG/tree)
Yes	80
No	35
Total	67

Table 12. ANOVA and significance statistics of the total cost of production between permanent and non-permanent irrigation.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	4E+008	1	390976377	1.774	
Within groups	2E+010	89	220351905		
Total	2E+010	90			

Table 13. Effect of source of irrigation on the cost of production of date fruits in the Northern state.

Source of irrigation	Cost (SDG/tree)
Nile	42
Underground	69
Total	53

Table 14. ANOVA of significance statistics of the total cost of production based on source of irrigation.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	1E+008	1	131286098	1.56	0.215
Within groups	6E+009	75	84056023		
Total	6E+009	76			

Table 15. Effect of the level of education on the cost of production of dates northern Sudan.

Level of education	Cost (SDG/tree)
Khalwa	31
Primary	103
Secondary	50
University	88
Illiterate	69
Total	69

Table 16. ANOVA and significance statistics of the total cost of production between different levels of education.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	1E+008	4	134532887	0.588	0.67
Within groups	2E+010	85	228700748		
Total	2E+010	89			

Table 17. Effect of the off-farm work on the cost of production of dates in Northern Sudan.

Off-farm work	Cost (SDG/tree)
Yes	70
No	65
Total	67

Table 18. ANOVA and significance statistics of the total cost of production and education.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	4094203	1	4094203	0.018	0.89
Within groups	2E+010	87	229434024		
Total	2E+010	88			

Table 19. Relationship between productivity and the cost of production of dates.

Productivity	Cost (SDG/tree)
Less than 50 kg/tree	55
More than 50 kg/tree	83
Total	64

Table 20. ANOVA and significance statistics of the cost of production for education.

	Sum of squares	df	Mean square	F	Sig. F
Between groups	2E+008	1	177353733	0.84	0.36
Within groups	2E+010	95	210014376		
Total	2E+010	96			

Eggs Distribution of Old World Bug (Dubas Bug) *Ommatissus lybicus* (Derbeg) Asche and Wilson (*Homoptera*: *Tropiduchidae*) on Fronds Rows and Effect of Dust Storms on Three Varieties of Date Palm Trees

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Abstract

Results of eggs distribution of *Ommatissus lybicus* on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') indicated that 'Zahdi' was the favorite variety for eggs laying in the field during three years between 2004 and 2006, while 'Daery' was the less favorite one. The study also showed that the eggs distribution was different depending on fronds rows. The population density of average eggs ratios laid on 1st and 2nd frond rows were 64.3 and 64.4%, respectively, compared with 18.8 and 15.8% on the 4th and the 5th, and the average of eggs ratios decreased at the apex raws. The eggs population density of the second generation was higher than the first generation. There are significant differences in eggs distribution laid ratios between the 1st and the 2nd rows in 2004. The average eggs ratios were 5.8 and 3.3%, respectively, during 2005, while the ratios were 20, 17.4, 18.1, and 16.4% in the 5th, 6th, 7th, and 8th, respectively. The eggs distribution on frond rows were different with natural distribution, the results also showed that continuous occurrence of dust storms during June and July 2005 was a harmful factor causing reduction of means egg on the 1st and 2nd to about zero and 17.4, respectively, in the first generation during 2006, while the (4-8) front rows had 33.9, 27.3, 13.4, 2.2 and zero egg/leaflet in Iraq during June and July 2005 affecting the insect females which laid unfertilized eggs on fronds rows.

INTRODUCTION

Date palm trees are infested by various kinds of agricultural pests, one of these pests being the dubas bug *Ommatissus lybicus* (Debergevin) Asche and Wilson. (*Homoptera*: *Tropiduchidae*) the nymphs and adults sucking the sap from leaflet, fruits and fruits stalks. The female makes a lengthy crack into leaflet tissues especially in the midrib of leaflets to lay eggs which cause the leaflet to die. The nymphs and adults consume the honey dew on the different parts of infested trees and encourage black mould fungus to grow, and also accumulate dust which causes the activity of infested trees to weaken, and trees infestated for more than three years show reduce production and finally die (Hussain, 1963, 1974; Jassim, 2007).

The insect has two generations during a year, one of them is winter and the other is summer (Dowson, 1936), while Al-Hafidh (1988, 2009) also mentioned that the insect had two generations, one of them being the spring generation and the other the autumn generation in lraq and the UAE. The spring generation starts in early April till the second week of June. The eggs are still in dormancy till the beginning of August and the spring generation disappears in the 3rd week of September, and the females lay their eggs inhomogeneously according to the kind of tree fronds rows. The eggs highest ratio is laid on the second row in the spring generation while in the autumn generation the eggs were laid on 4th frond row while the female never laid eggs on 7-10 rows (Hussain, 1974, 1985).

Hasson (1988) studied the biology of insects in lab conditions and at critical temperature of growth for each insect stage, and considered 25 and 30°C were suitable temperatures for egg nursing which lasts 42.42 and 42.15 days, respectively.

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Al-Kafaji and Jasim (1992) declared that the insect population density of the moving stage was correlated with four directions of the tree, and the numbers of insect individuals were significantly different according to the tree direction and the highest populations were into northern and then into southern directions. The highest mean of insect eggs, which were laid by females was during the middle of July for the summer generation and mid-November for the autumn generation. The eggs are still in dormancy till the beginning of April (Al-Kafaji et al., 1995), while Jassim (2007) mentioned that the highest means of eggs were always inserted in the midrib of upper leaflet surface in the first generation, while they were laid in the blade of upper and lower leaflets surfaces just close to the midrib in the second generation, declaring that the most was laid on the first row of the frond, which makes it easy to get rid of this frond row in the mechanical control of the insect.

Ba-Angood et al. (2009) studied the insect biology in Hadramout in Yemen. The insect had two generations per year, autumn and spring generation. The spring starts in the third week of January, while autumn generation starts in the first week of September, when the temperature ranges between 28-30°C.

Abdul et al. (2009) registered the insect dispersal in Al-Anbar province in Iraq in the spring of 2007 and mentioned that the insect infestation had not been seen, and that it had been seen in the south of Heet city during the autumn generation.

The investigators disagree with eachother about the egg distribution on fronds rows and mentioned that the egg distribution depends on the insect generation. For this reason, this study aimed to investigate insect dispersal and eggs distribution for future applications.

MATERIALS AND METHODS

Study of Population Density of Eggs and Their Distribution in the Leaflet

One orchard was chosen in Rashidia region (North Baghdad). The orchard contained various date palm varieties and they were highly infested by dubas bug. Three varieties were chosen as famous varieties ('Zahdi', 'Khustawi' and 'Daery'), the three varieties were planted randomly. The trees were nearly similar to each other in size (16 trees) for each variety. After the trees had been numbered, random samples were taken from several fronds rows in order to estimate the infestation correctly. All the samples were tested in a lab. To count the number of laid eggs means each leaflet for three varieties and replicates was taken, the trees number were signed to representive sample size randomy according to the Morris (1955) hypothesis with standard error up to 10%. The relative variance was counted according to the following equation (Norris et al., 2003):

$$[RV = (S_{x^-} / \bar{X})(100)]$$

RV = Relative variance
S_{x^-} = mean standard settlement
$\bar{X}$ = Settlement termedice

Egg Distribution on Fronds Rows

Four replicates of each variety were chosen. Each replicate represented one tree that contained the same numbers of fronds rows. The trees which were chosen had 10 fronds rows and more, while the grown apex rows which contained more fronds rows were left in the place of study region investigated. The fronds were cut from the base according to each raw, each frond was divided into three parts (apex, median and basal) according to the median vein length, and two parts one of them, left and right from the frond median vein, ten leaflets from each part were taken alternately. In total 30 leaflets were taken for each frond, the total leaflet number for each replicate which was to be tested was about 1200 leaflets. The egg numbers were registered and put into special tables and the eggs distribution on fronds rows for 5 generations during the years 2004, 2005 and 2006 were studied.

RESULTS AND DISCUSSION

Results of Table 1 show that the egg population density which was laid by insect females for the second generation for three date palm varieties varied significantly during 2004 according to the various fronds rows and tree variety. The first and second rows were appropriated the ratio of about 64% for eggs laid. The eggs mean ratios laid on the first frond row were 1549.8, 1114.8 and 645.8% eggs/leaflet for 'Zahdi', 'Khistawi' and 'Daery', respectively, while in the second frond raw they were 852.8, 933.5 and 522.8% eggs/leaflet, respectively.

Table 1 also shows that the eggs mean laid were reduced in the apex tree frond rows, especially in the 7^{th} and 8^{th} rows, the reason probably depending on leaflet direction to the top of tree which exposes the eggs to the cooled air during winter in addition to the fallen rain directly. The other reason may be the fine and smooth midrib of the leaflet, the lower fronds rows were too hard and more ribbed which offers better protection for dormant eggs. These results disagree with the results of Hussain (1985) who found that the highest mean population density of eggs were laid on the second frond row.

Table 1 indicates that the total eggs laid on different fronds rows of three tree varieties were various. The mean eggs laid on 'Zahdi' and 'Khustawi' were 467.2 and 407.8 eggs/leaflet, respectively, while this was 215.6 eggs/leaflet for 'Daery' which can be consider unfavorable for females to lay their eggs. The insect females laid about ⅔ from the total eggs laid on the first and second frond rows. This hypothesis should serve to control the insects mechanically by cutting and burning these two raws to get rid of the infection source, which is considered as a good alternative control method to chemicals.

Table 2 indicates that the population density of eggs laid on eight frond rows for the three varieties in the second generation were varied significantly. The eggs mean laid increased in amount when compared with the amount of the first generation. Also, there was a big variation of eggs mean laid in the first and second frond rows, especially for 'Khustawi' and 'Daery', which were 4918 and 1740 eggs/leaflet, respectively, compared with 1603.5 and 1355.3 eggs/leaflet, respectively, in the first frond row, while the eggs mean laid in the first frond row was 3042.3 eggs/leaflet for 'Zahdi' and 2398.8 eggs/leaflet in the second raw. The eggs mean laid ratio for both rows was 75.3% which reduced gradually at the apex of the tree to become 0.8%. This decrease may be a result of sun and heating air effects during June, July and August.

The results of the study disagree with the results found by Hussain (1974) who mentioned that the highest eggs population density for the second generation (in autumn) was in the 4^{th} frond row. Also disagree with them: the new frond rows between 7-10 for the second generation have no eggs laid by insect females as a result of the effect of temperature, humidity and wind velocity and orchord caring.

Table 2 also shows that the eggs laid mean on different raws varied according to the different tree variety. The means were 991.3, 931.4, and 574.1 eggs/leaflet, respectively. 'Zahdi' was more preferred by insect females than 'Khustawi', while 'Daery' was the least preferred for eggs laid. Results of statistical analyses declared there is no significant difference between 'Zahdi' and 'Khustawi', but there are significant differences between 'Daery' and both 'Zahdi' and 'Khustawi', especially in the first and second row.

Table 3 indicates that the female insect behavior in eggs laying did not differ in 2004 compared with the same behavior in 2005 for the same generation. The female also laid most of the eggs on the first and second rows and decreased gradually at the rows top in three varieties but the general mean of eggs laid decreased in 2005, compared with the previous year. Egg numbers laid on the first and second frond rows differ in an irreversible way for 'Zahdi' and 'Daery'. The eggs means of the second frond raw were 1569.5 and 488.5 eggs/leaflet, respectively, while they were 817.0 and 371.3 eggs/leaflet, respectively, in the first frond row. The egg mean for 'Khustawi' was 615 eggs/leaflet in the first frond row and 553.0 eggs per leaflet in the second row. On the other hand, the egg laid ratios on eight frond rows were various for the three varieties, being 26.3, 38.1, 14.4, 10.1, 5.7, 4.9, 0.7 and 0.4, respectively.

Table 4 indicates that the total eggs means laid were various according to the kind of variety. The amounts eggs laid were 423.5, 245.1, and 189.4 eggs/leaflet for the three varieties, respectively. The statistical analysis results pointed to significant differences between the three varieties.

The study found that the climate can be considered as a vital factor affecting the insect female behavior in eggs distribution on frond rows for the three different varieties during 2005. The eggs distribution completely differed compared with the natural distribution that most investigators mentioned in their studies. Continuous occurrence of dust storms during June and July 2005 affected insect females and the eggs laying. The females laid their eggs on fronds rows randomly.

Table 4 also shows the abnormal distribution of eggs laid, the 4-8 front rows had the highest amount of eggs laid compared with the first and second rows because the upper rows are directly exposed and receive more moist compared to the inner rows. The eggs that were laid in 'Zahdi' and 'Daery' in inner rows were 42.0 and 146.8 eggs/leaflet, respectively, while they were 709.5 and 872.5 eggs/leaflet in 'Khustawi'. The general eggs mean laid was 620 eggs/leaflet in 'Daery' for the second generation. Results of the study completely agree with the results of Hubaishan et al. (2005). The absence of natural enemies and chemical control during 2003, 2004, and 2005 caused the outbreak in population density of insects (Jassim, 2007).

The results in Table 5 show that the general mean of eggs laid on different fronds rows for the three varieties in the first generation of 2006 were the lowest when compared to the general mean of the same generation during 2004 and 2005. The means during 2006 were 54.8, 40.8, and 21.3 eggss/leaflet, respectively. The cause of this low mean was a reduction of most adult insects under the effect of dust storms. The mode of eggs distribution of the first generation varied completely with the eggs distribution modes of the previous years. The insect females did not lay any eggs on the first front row, while the eggs distribution ratios on the front rows between 2-8 were 5.4, 17.6, 33.9, 27.3, 13.4, 2.2 and 0 eggs/leaflet, respectively.

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Tables

Table 1. Eggs distribution of dubas bug *O. lybicus* on fronds rows in first generation on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') in the Rashidia area in Baghdad province during 2004.

Variety	Means number of eggs/leaflet in the fronds rows								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Means
Zahdi	1549.8	852.8	486.8	511.3	249.8	79.7	8.0	0.0	467.2
Khustawi	1114.8	933.5	435.5	569.0	149.5	50.8	6.0	3.5	407.8
Daery	645.8	522.8	310.8	70.8	97.3	78.0	0.0	0.0	215.6
Mean	1103.3	769.7	411.03	383.7	165.5	69.5	4.6	1.16	
Percentage	37.9	26.4	14.1	13.1	5.7	2.4	0.17	0.03	

LSD 0.05 for V = 133.02
LSD 0.05 for S = 217.22
LSD 0.05 for V*S = 632

Table 2. Eggs distribution of dubas bug *O. lybicus* on fronds rows in the second generation on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') in the Rashidia area in Baghdad province during 2004.

Variety	Means number of eggs/leaflet in the fronds rows								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Means
Zahdi	3042.3	2398.8	517.5	923.5	514.5	360.0	110.3	63.0	991.2
Khustawi	1355.3	4918.0	228.3	335.3	221.3	2.1	94.0	98.3	931.4
Daery	1603.5	1740.0	670.0	329.8	143.8	76.3	21.0	8.5	474.1
Mean	2000.3	3018.9	471.9	529.5	293.2	212.4	75.1	56.6	
Percentage	30.0	45.3	7.8	7.9	4.4	3.1	1.1	0.8	

LSD 0.05 for V = 133.02
LSD 0.05 for S = 217.22
LSD 0.05 for V*S = 632

Table 3. Eggs distribution of dubas bug *O. lybicus* on fronds rows in the first generation on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') in the Rashidia area in Baghdad province during 2005.

Variety	Means number of eggs/leaflet in the fronds rows								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Means
Zahdi	817.0	1569.5	408.0	240.0	176.3	154.0	22.0	2.3	423.5
Khustawi	615.0	553.0	356.5	300.8	72.0	63.3	0.0	0.0	245.1
Daery	371.3	488.5	226.8	146.5	142.0	117.5	22.8	0.0	189.4
Mean	600.8	870.3	330.4	229.1	130.1	111.6	14.9	0.8	
Percentage	26.3	38.1	14.4	10.1	5.7	4.9	0.7	0.04	

LSD 0.05 for V = 137.39
LSD 0.05 for S = 224.35
LSD 0.05 for V*S = 578.1

Table 4. Eggs distribution of dubas bug *O. lybicus* on fronds rows in the second generation on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') in the Rashidia area in Baghdad province during 2005.

Variety	Means number of eggs/leaflet in the fronds rows								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Means
Zahdi	0.0	0.0	504.3	1364.5	1653.3	978.5	838.3	723.3	
Khustawi	709.5	872.5	236.3	716.5	1253.0	960.8	780.8	959.5	811.1
Daery	42.0	146.8	187.3	187.5	630.3	1138.3	1407.3	1220.8	620.0
Mean	250.5	339.8	309.3	756.2	1178.9	1025.9	1068.8	967.9	
Percentage	4.3	5.8	3.3	12.8	20.0	17.4	18.1	16.4	

LSD 0.05 for V = 137.39
LSD 0.05 for S = 224.35
LSD 0.05 for V*S = 578.1

Table 5. Eggs distribution of dubas bug *O. lybicus* on fronds rows in the first generation on three varieties of date palm trees ('Zahdi', 'Khustawi' and 'Daery') in the Rashidia area in Baghdad province during 2006.

Variety	Means number of eggs/leaflet in the fronds rows								
	1st	2nd	3rd	4th	5th	6th	7th	8th	Means
Zahdi	0.0	0.0	88.3	166.8	118.3	48.3	13.8	3.25	54.8
Khustawi	0.0	47.5	60.0	101.8	738.0	42.8	0.8	0.0	40.8
Daery	0.0	4.8	15.0	49.5	62.8	33.3	4.8	0.0	21.3
Mean	0.0	17.4	54.4	106	84.9	41.4	6.5	0.0	
Percentage	0.0	5.4	17.6	33.9	27.3	13.4	2.2	0.0	

LSD 0.05 for V = 7.2
LSD 0.05 for S = 11.7
LSD 0.05 for V*S = 20.2

The Use of Different Insect Control Regimes Using Three Green Chemicals to Combat *Viracola livia* on Date Palm Fruit in Egypt

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Keywords: field trials, spinosad, spinetoram, methoxyfenozide, IPM

Abstract

Seven different insect control regimes comprising of three green chemicals: two spinosyns, spinosad (Tracer®24 SC) and spinetoram (Radiant®12 SC), and the insect growth regulator (moulting hormone agonist) methoxyfenozide (Runner®24 SC) were employed to control the pomegranate butterfly *Viracola livia* on date palm fruit in 2006/7. Each spray regime consisted of two applications per season applied at a 3 week interval.

The damage was very severe with larvae penetration reaching 99 and 85% in the untreated in 2006 and 2007, respectively, two weeks prior to harvest. At this assessment, the results illustrated that spinetoram at 2.4 g ai/100 L sprayed twice/season was equivalent to spinosad at 4.8 g ai/100 L with the same spray frequency, and was also on par when spinosad was followed with different rates of methoxyfenozide. However, at harvest time, the spinetoram rate showed significantly better results than spinosad, indicating that there is at least a two-fold better activity of spinetoram than spinosad on a rate to rate comparison. Alternation of spinosad at 4.8 g ai/100 L followed by methoxyfenozide at 3.6-6.0 g ai/100 L was equivalent with spinetoram. It is clear that both spinosyns, applied alone, are very effective to control the pomegranate butterfly, with spinetoram exhibiting a 2-fold greater activity than spinosad. The application of spinosad in rotation with methoxyfenozide is in line with the IPM strategy for management of date pests. All products proved to perform well under dry conditions and high temperatures (reaching 51°C) without any phytotoxicity symptoms either on the fruit or the tree foliage.

INTRODUCTION

Date palm is considered one of the most important cash crops in the New Valley Governorate. In this Governorate more than one million date palm trees are grown. Besides the local consumption, dates are also exported to foreign countries. The pomegranate butterfly, *Viracola livia* (Klug.) is considered the most important late insect pests attacking fruit before harvest.

The pomegranate butterfly *V. livia* is serious pest attacking date palm as well as pomegranate fruit trees in Egypt. Infestation of this pest has a direct effect on the exportation value due its rot fermentation of the fruits. To combat this pest in Egypt, conventional insecticides as malathion or carbaryal were used (Awadalla et al., 1970; Hussein and Gouhar, 1972; Abd El-Rahim et al., 1974).

Due to environmental toxicity the Ministry of Agriculture (MOA) banned all conventional insecticides from use on dates. They allowed only bio-insecticides or organic or safe products. Currently available bio-insecticides are slow acting and do not satisfy farmers' need (Sayed et al., 2001). Farmers started to use bio-products (*Bacillus thuringiensis*) but due to the very poor performance they stopped using these products.

Spinosad acts quickly and has a speed of kill comparable to most synthetic insecticides. It acts significantly faster than slow acting products like *Bacillus*, *Beauvaria* and other traditional biologicals (Bret et al., 1997).

The Environmental Protection Agency (EPA) awarded Spinosad (Tracer) and Methoxyfenozide (Runner) the reduced risk product category in 1997 and 2000. Spinosad

was considered as organic in the USA and the EU in 2002 and 2008, respectively. Spinetoram is the 2nd generation of spinosad. It came as fermentation from the same soil bacteria *Saccharopolyspora spinosa* Mertz and Yao. The Environmental Protection Agency (EPA) awarded the green chemistry challenge award for Runner (confirm), Spinosad and Spinetoram in 1998 1999 and 2009, respectively. These three green chemicals were utilized in 7 different regimes to combat *V. livia*.

MATERIAL AND METHODS

Programs used for both insect targets:

1. Radiant/radiant = at 10 ml, then again at same rate, 3 weeks interval.
2. Radiant/radiant = radiant at 15 ml, then again at same rate, 3 weeks interval.
3. Radiant/radiant = radiant at 20ml, then again at same rate, 3 weeks interval.
4. Tracer/tracer = Tracer at 20 ml, then again at same rate, 3 weeks interval.
5. Tracer/runner A = Tracer at 20ml, runner at 15ml, 3 weeks interval.
6. Tracer/runner B = Tracer at 20ml, runner at 20ml, 3 weeks interval.
7. Tracer/runner C = Tracer at 20ml, runner at 25ml, 3 weeks interval.

In 2006/7 field trials were conducted in El-Dhakla Oasis to evaluate 7 different insect control regimes to combat the pomegranate butter fly.

The following treatments were applied as follows:

1. Tracer 24% SC (Spinosad) at the recommended rate of 20 ml/100 L. Tracer 24 SC, is a trademark of the Dow Agrosciences Co. containing 240 active ingredient as spinosad (Spinosyn A and D). It is a natural metabolite of the actinomycete, *Saccharopolyspora spinosa* Mertz and Yao.
2. Radiant 12 SC (Spinetoram) at 10, 15 and 20 ml/100 L. Radiant 12SC, is a trademark of the Dow Agrosciences Co. containing 120 active ingredient as spinosad (Spinosyn J and L). It is derived from fermentation of the actinomycete, *Saccharopolyspora spinosa* Mertz and Yao and followed by chemical modification to create the unique active ingredient in spinetoram.
3. Runner 24% SC (Methoxyfenozide), at 15, 20 and 25 ml/100 L. Runner, is a trademark of the Dow Agrosciences Co. containing 240 active ingredient as Methoxyfenozide. It is an insect growth regulator (IGR) as moult accelerating compound.

The date palm variety was 'Saidi' and date palms in all experiments were almost the same height. All treatments were replicated three times. One date palm tree was considered as one replicate. The samples' size was 10 strands/one date palm taken at random from each replicate 3 weeks after any spray and on 30th August before harvest. The first spray was done on 30/6 and the second spray three weeks later. A ground motor of 600-L volume was used

Assessment

The number of fruits/30 strands, the total number of eggs found on fruits, the number of hatched eggs found, the number of fruits having alive larvae, infestation of fruit % and reduction % were done/each treatment. Unhatched eggs were counted before harvest sample only in order to be sure that enough time was given for possibility of hatching.

Successful penetration was calculated based on the number of hatched eggs found on fruit and the number of alive larvae succeeded in penetration.

Last inspection was at mid September (during harvest) of each year. Samples size was 100 fruit/one date palm taken at random from each replicate. Replicates were 3 date palm trees. Infested fruit dates were counted. Statistical analysis was done for infestation figures which turned then after to reduction % based on Abbot formula.

Meteorological conditions were recorded during the trials.

RESULTS AND DISSCUSION

Seven different insect control regimes including old and new generation of spynosin to combat *Viracola livia* on date palm trees were used in 2006/7. The old

spynosin was Spinosad (Tracer 24 SC) and the new generation was spinertoram (Radiant 12 SC).

Tables 1 and 2 show the reduction % of *V. livia* infestation after fruit being treated by 7 different regimes in 2006 and 2007.

Before harvest, infestation % of alive larvae into the fruit by using radiant at 20 ml/100 L sprayed twice was equal with Tracer at 20 ml/100 L sprayed twice in 2006 and 2007, respectively. The last was equal with Tracer 24 SC at 20 ml then Runner at 15 ml/100 L. Zero alive larvae were recorded inside fruit for Radiant 12 SC at 20 ml sprayed twice/season as well as tracer 24 SC at 20 ml then Runner 24 SC at 25 ml/100 L in 2006 and 2007, respectively. However, it reached 212 out of 566 fruit in 2006 and 350 out of 500 fruit in 2007. Successful penetration % reached to 99% and 85% in the untreated in 2006 and 2007, respectively. The last reflect the excellent effect on the neonate larvae. Nolting et al. (1997) stated that Spinosad has a good ovi-larvicide activity.

At harvest time, Radiant 12 SC at 20 ml/100 L sprayed twice showed significantly better results than tracer 24 SC at 20 ml/100 L sprayed twice. The last proved that Radiant was more than 2.0-fold stronger than tracer, especially when active % was considered. A similar trend of results between the two spynosins was indicated by Temerak (2007) when cotton leaf worm was used as target.

Spinosad (or spinetoram) is activating the nicotinic acetylcholine receptors at special sites. It effects ion currents through Gamma-Aminobutyric Acid (GABA). It represents a unique mode of action (Salgado, 1997). The availability of a novel chemical group, with a new mode of action that is different from insecticides in current use, is an asset to insecticide resistance management programs (Kranthi et al., 2000). Temerak (2003) indicated that Spinosad was not easily affected by the existing resistance mechanism.

Methoxyfenozide (Runner) imitates the action of the moulting hormone at its receptor. So, it inhibits the synthesis of the new exoskeleton (Salgado, 1997).

One key pest management strategy is the rotation of different mode of action to ensure that continual selectivity for resistance does not occur and the possibility of pest resistance is avoided or at least significantly delayed (Thompson et al., 1997; Temerak 2002). Application of tracer in rotation with runner is an excellent example of a functional date palm integrated pest management program.

Alternation tracer at 20 ml as a basic with the lowest rate of runner as 15 ml/100 L as 2nd spray showed the same efficacy as the maximum rate of runner. The last was equal with Radiant at 20 ml/100 L sprayed twice/season. Application of tracer in rotation with runner is an excellent example of a functional date palm integrated pest management program.

Tables 3 and 4, are representing the metrological condition during the trials for *V. livia*. All products proved to be working well under dry conditions with high temperatures reaching 51°C. Tracer was working under 51°C without phytotoxicity (Temerak and Sayed, 2001; Sayed and Temerak, 2003).

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Table 1. Reduction % of *Viracola livia* infestation after fruits being treated with different rotation programs, New Valley, Egypt, 2006.

Criteria	Treatments/100 L							
	Radiant /Radiant 1	Radiant /Radiant 2	Radiant /Radiant 3	Tracer /Tracer 4	Tracer /Runner 5	Tracer /Runner 6	Tracer /Runner 7	Cont.
	1st Spray 30/6/2006 - Evaluation after 3 weeks							
No. of fruits/30 strands	560	499	465	460	518	450	447	590
Total no.eggs found	125	155	145	200	135	114	88	244
No. hatched eggs found	90	85	70	110	90	65	68	151
No.fruit with alive larvae	21.1	16	16	19	22	13	14	57
Successful penetration %	15.2	18.8	22.8	17.2	24.4	20	20.5	37.3
Infestation of fruit %	3.3	3.2	3.4	3.9	4.2	2.8	3.1	9.6
Reduction %	65.6	66.6	64.5	59.3	56.2	70.8	67.7	-
	2nd Spray of 21/7/2006 - Evaluation after 3 weeks							
No. of fruits/30 strands	550	543	545	579	525	546	499	500
Total no.eggs found	220	188	233	210	232	173	156	244
No. hatched eggs found	7	4	88	1	94	99	148	226
No.fruit with alive larvae	0	5	1	0	3	0	0	209
Successful penetration %	0	0.2	1.13	0.4	3.19	0	0	92.47
Infestation of fruit %	0	0.9	0.18	0	0.49	0	0	33.8
Reduction %	100	97.3	99.46	100	98.55	100	100	-

Table 1. Continued.

Criteria	Treatments/100 L							
	Radiant /Radiant 1	Radiant /Radiant 2	Radiant /Radiant 3	Tracer /Tracer 4	Tracer /Runner 5	Tracer /Runner 6	Tracer /Runner 7	Cont.
	Before harvest - Evaluation 30/8/2006							
No. of fruits/30 strands	536	555	560	540	523	549	525	566
Total no. eggs found	205	199	175	188	220	181	175	217
No. hatched eggs found	196	192	169	183	212	176	171	215
Un hatched eggs %	4.3	3.5	3.4	2.6	3.6	2.7	2.2	0.9
No. fruit with alive larvae	2	7	0	5	2	1	2	212
Successful penetration %	1	3.6	0	2.7	0.9	0.5	1.1	98.6
Infestation of fruit %	0.3 *ab*	1.2 *b*	0 *a*	0.9 *ab*	0.3 *ab*	0 *a*	0.30 *ab*	33.3 *c*
Reduction %	99.0	96.3	100	97.2	99.0	100	99.0	-
	During harvest 15/9/2006							
Infestation %	6.8 *c*	6.9 *c*	0 *a*	4.3 *b*	1.0 *a*	0.66 *a*	0.66 *a*	69.0 *d*
Reduction %	90.1	90.0	100	93.7	99.0	98.5	99.0	-
LSD 0.05	1.02							

Figures followed by the same letter are not significantly different.
1. Radiant/radiant = at 10 ml, then again at same rate, 3 weeks interval.
2. Radiant/radiant = radiant at 15 ml, then again at same rate, 3 weeks interval.
3. Radiant/radiant = radiant at 20 ml, then again at same rate, 3 weeks interval.
4. Tracer/tracer = Tracer at 20 ml, then again at same rate, 3 weeks interval.
5. Tracer/runner A = Tracer at 20 ml , runner at 15 ml, 3 weeks interval.
6. Tracer/runner B = Tracer at 20 ml , runner at 20 ml, 3 weeks interval.
7. Tracer/runner C = Tracer at 20 ml , runner at 25 ml, 3 weeks interval.

Table 2. Reduction % of *Viracola livia* infestation after fruits being treated with different rotation program, New Valley, Egypt, 2007.

Criteria	Treatments / 100 L							
	Radiant /Radiant 1	Radiant /Radiant 2	Radiant /Radiant 3	Tracer /Tracer 4	Tracer /Runner 5	Tracer /Runner 6	Tracer /Runner 7	Cont.
	1st Spray 30/6/2007 - Evaluation after 3 weeks							
	428	478	571	473	463	552	529	
No. of fruits/30 strands	222	249	231	246	248	188	168	486
Total no.eggs found	188	219	180	210	189	117	151	183
No. hatched eggs found	13	3	10	14	13	10	8	142
No.fruit with alive larvae	6.9	1.3	5.5	6.6	6.8	8.5	5.2	26
Successful penetration %	3.0	0.6	1.7	2.9	2.8	1.8	1.5	18.3
Infestation of fruit %	43.3	88.6	67.9	45.2	47.1	66.0	71.6	5.3
Reduction %								-
	2nd Spray of 21/7/2007 - Evaluation after 3 weeks							
No. of fruits/30 strands	479	509	540	518	520	557	571	480
Total no.eggs found	229	345	285	361	316	397	481	272
No. hatched eggs found	190	265	247	290	241	338	415	135
No.fruit with alive larvae	1	1	1	1	0	0	2	123
Successful penetration %	0.5	0.3	0.4	0.3	0	0	0.4	98.4
Infestation of fruit %	0.2	0.19	0.18	0.19	0	0	0.3	25.6
Reduction %	99.2	99.2	99.2	99.2	100	100	98.8	-

Table 2. Continued.

Criteria	Treatments / 100 L							
	Radiant /Radiant 1	Radiant /Radiant 2	Radiant /Radiant 3	Tracer /Tracer 4	Tracer /Runner 5	Tracer /Runner 6	Tracer /Runner 7	Cont.
	Before harvest - Evaluation on 30/8/2007							
No. of fruits/30 strands	450	500	597	397	450	590	456	500
Total no. eggs found	350	340	247	174	287	278	235	413
No. hatched eggs found	341	335	245	156	287	276	230	413
Un hatched eggs %	2.5	1.4	0.8	10.3	0	0.7	2.1	0
No. fruit with alive larvae	3	7	2	4	3	2	0	350
Successful penetration %	0.87	2.0	0.8	2.56	1.0	0.7	0	84.7
Infestation of fruit %	0.6 *ab*	1.4 *b*	0.3 *ab*	1.0 *ab*	0.6 *ab*	0.3 *ab*	0 *a*	70 *c*
Reduction %	99.1	98.0	99.5	98.5	99.1	99.5	100	-
	During harvest 15/9/2007							
Infestation %	7.1 *c*	7 *c*	0.3 *a*	4.8 *b*	1.3 *a*	1 *a*	0.6 *a*	73.3 *d*
Reduction %	90.3	90.4	99.5	93.4	98.2	98.6	99.1	-
LSD 0.05	1.111							

Figures followed by the same letter are not significantly different.
1. Radiant/radiant = at 10 ml, then again at same rate, 3 weeks interval.
2. Radiant/radiant = radiant at 15 ml, then again at same rate, 3 weeks interval.
3. Radiant/radiant = radiant at 20ml, then again at same rate, 3 weeks interval.
4. Tracer/tracer = Tracer at 20 ml, then again at same rate, 3 weeks interval.
5. Tracer/runner A = Tracer at 20 ml , runner at 15 ml, 3 weeks interval.
6. Tracer/runner B = Tracer at 20 ml , runner at 20 ml, 3 weeks interval.
7. Tracer/runner C = Tracer at 20 ml , runner at 25 ml, 3 weeks interval.

Table 3. Some meteorological conditions during the trials of *V. livia* and all products (Tracer, Radiant, and Runner), New Valley, Egypt, 2006.

Criteria	*V. livia*		
	July	Aug.	Sept.
Max. temp.(°C)	47	51.1	44.1
Aver. max. temp.(°C)	41.1	42	40
Aver. Min. temp. (°C)	27.1	27.6	26.2
Aver. max. RH (%)	29.9	35	43.9
Aver. min. RH (%)	11	12	13

Altitude: 72 m; Longitude: 30°34'; Latitude: 25°26'.

Table 4. Some meteorological conditions during the trials of *V. livia* and all products (Tracer, Radiant, and Runner), New Valley, Egypt, 2007.

Criteria	*V. livia*		
	July	Aug.	Sept.
Max. temp.(°C)	45.9	51.4	44.4
Aver. max. temp.(°C)	41.5	41	39.9
Aver. Min. temp. °C)	27.3	27.8	26.1
Aver. max. RH (%)	31	36	45
Aver. min. RH (%)	12	12	14

Altitude: 72 m; Longitude: 30°34'; Latitude: 25°26'.

The Use of Different Insect Control Regimes Using Three Green Chemicals to Combat *Batrachedra Amydraula* Meyrick and *Cadra* spp. on Date Palm Fruit in Egypt

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Abstract

Seven different insect control regimes comprised of three green insecticides were employed to combat two date pests; the lesser date moth *Batrachedra amydraula* Meyrick and the almond moth *Cadra* (*Ephestia*) spp. on date palm fruit in 2006-07. The chemicals tested were the two spinosyns, spinosad (Tracer® 240SC) and spinetoram (Radiant® 120SC), and the insect growth regulator (moulting hormone agonist) methoxyfenozide (Runner® 240SC). Each spray regime consisted of two applications per season applied 2 to 3 weeks apart.

***Batrachedra amydraula* fruit infestation ranged from 17.2 to 24.2% after six weeks and the spray regime of two applications at a 14 day interval per season showed that spinosad at 4.8 g ai/100 L was equivalent to spinetoram at 1.8 g ai/100 L in both years of the study. This indicated that spinetoram was close to 2.7-fold more active than spinosad against this pest based on a grams active equivalence. Alternating spinosad with methoxyfenozide as a second spray showed similar efficacy regardless of the rate of methoxyfenozide (3.6 to 6.0 g ai/100 L).**

Fruit infestation was lower for *Cadra* spp. at harvest time (7.7 to 14%), but the results showed that spinetoram at 1.2 g ai/100 L was equivalent to spinosad at 4.8 g ai/100 L, indicating that spinetoram was 4-fold more active than spinosad on a gram active basis when sprayed twice per season at a 21 day interval. For the almond moth, a similar spray regime can be recommended as with the lesser date moth, applying spinosad at 4.8 g ai/100 L then methoxyfenozide at 3.6 g ai/100 L.

For both lepidopterous date pests, it is clear that both spinosyns are very effective applied alone, and spinetoram is 2.7- to 4-fold more active than spinosad. The application of Tracer followed with Runner is an excellent example of a functional date palm integrated pest management program.

All products performed well under dry conditions with high temperatures reaching 51°C without any phytotoxicity symptoms either on the fruit or the tree foliage.

INTRODUCTION

Date palm is considered one of the most important cash crops in the New Valley Governorate in Egypt, where there are more than one million date palm trees. Besides the local consumption, dates are also exported to foreign countries. The lesser date moth, *Batrachedra amydraula* Meyrick, is the most serious early insect pest on date fruit in the Oases of the New Valley (Saleh 1974; Badawi et al., 1977). It is also considered a key insect pest in surrounding countries, e.g., Israel, Iraq, Iran, Yemen, Libya, Saudi Arabia and the Emirates. The loss due to this pest only can reach 30% of the final yield. The larvae attack young date fruit which then usually stop growing. Larvae of this pest feed on the flesh along the stone in May and June, causing most of the fruit to become red-brown and finally drop (Badawi et al., 1977; Venezian and Blumberg, 1982). Blumberg

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(1975) observed considerable fruit drop of dates by *B*. *amydraula*, mainly between April and June in Egypt and Israel, respectively. Saleh (1974) stated that infestation of *B*. *amydraula* starts from the first of July onwards, and decreases gradually towards the end of August in the New Valley Governorate.

Cadra spp. (*C. cautella* and *C*. *calidella*) are important insect pests that start later in the season and continue in storehouses (Sayed and El-Deeb, 1996). Sayed and El-Deeb (1996) indicated that sex pheromones play a significant role for control inside the storehouse, but not in the field. Essa (2003) reported that satisfactory control was achieved by covering date bunches during the first half of July.

Spinosad is a natural metabolite of the soil actinomycete, *Saccharopolyspora spinosa*, and was approved as an organic product in the USA and the EU in 2002 and 2008, respectively. Spinetoram is derived from the semi-synthetic modification of other naturally-derived metabolites produced by this bacterium. Both products belong to the new chemical class known as spinosyns. Spinosad and spinetoram work by activating nicotinic acetylcholine receptors at a unique site (Salgado, 1997). Methoxyfenozide imitates the action of the moulting hormone at its receptor, and thereby it disrupts the synthesis of the new exoskeleton (Salgado, 1997). The US Environmental Protection Agency (EPA) awarded the Green Chemistry Challenge Award for the moult accelerating compounds (MAC's) that include methoxyfenozide in 1998, spinosad in 1999, and spinetoram in 2008. EPA also registered these three products under its reduced risk insecticide program. The availability of a novel chemical group as spinosyns, with a new mode of action that is different from insecticides in current use, is an asset to insecticide resistance management programs (Kranthi et al., 2000). Temerak (2003) also indicated that spinosad was not easily affected by existing resistance mechanisms.

Due to concerns about environmental toxicity, the Ministry of Agriculture in Egypt banned all conventional insecticides from use on dates. They allowed only bio-insecticides, organic, or those they characterized as 'safer' products. Current available bio-insecticides are slow acting and do not meet farmers' need (Sayed et al., 2001). Farmers started to apply bio-products (e.g., *Bacillus thuringiensis*) but due to the very poor performance farmers stopped using these. The speed of action of spinosad is comparable to most synthetic insecticides, and has been shown to act significantly faster than *Bacillus*, *Beauvaria*, and other traditional biologicals (Bret et al., 1997).

When there is more than one spray, it is preferable to use different safe and effective insecticides as part of a rotational program. Rotating products from different chemical classes with different modes of action helps to minimize or delay the risk of resistance. The current study examined the efficacy of seven different spray programs utilizing three green products to combat *B*. *amydraula* and *Cadra* spp.

MATERIAL AND METHODS

Three products were used in both years of testing on the two date pests:

1. Tracer 240SC applied at the recommended rate of 4.8 g ai/100 L (20 ml product/100 L). Tracer 240SC is a trademark of Dow AgroSciences and contains 240 g of spinosad per liter.
2. Radiant 120SC applied at 1.2, 1.8 and 2.4 g ai/100 L (10, 15 and 20 ml product/100 L, respectively). Radiant 120SC is a trademark of Dow AgroSciences and contains 120 g of spinetoram per liter.
3. Runner 240SC applied at 3.6, 4.8 and 6.0 g ai/100 L (15, 20 and 25 ml product/100 L, respectively). Runner is a trademark of Dow AgroSciences contain 240 g of methoxyfenozide per liter.

***Batrachedra amydraula* Treatments:**

1. Radiant at 1.2 g ai/100 L (10 ml product/100 L), applied twice.
2. Radiant at 1.8 g ai/100 L (15 ml product/100 L), applied twice.
3. Radiant at 2.4 g ai/100 L (20 ml product/100 L), applied twice.
4. Tracer at 4.8 g ai/100 L (20 ml product/100 L), applied twice.

5. Tracer at 4.8 g ai/100 L (20 ml product/100 L), followed by Runner at 3.6 g ai/100 L (15 ml product/100 L).
6. Tracer at 4.8 g ai/100 L (20 ml product/100 L), followed by Runner at 4.8 g ai/100 L (20 ml product/100 L).
7. Tracer at 4.8 g ai/100 L (20 ml product/100 L), followed by Runner at 6.0 g ai/100 L (25 ml product/100 L).

For all treatments, the second application was made 2 weeks after the first application.

In 2006 and 2007, field trials were conducted in El-Sherka 55 Village, Kharga Oasis to evaluate the different spray programs to control the lesser date moth, *Batrachedra amydraula*, on date palm fruit. The first spray was carried out in the last week of April and the second spray two weeks later. A motorized ground sprayer with a 600-L spray volume was used. The date palm variety was 'Saidi'. All treatments were replicated three times. One date palm tree was considered as one replicate. Samples size was 10 strands per one date palm taken at random from each replicate.

Fruit inspections were conducted at 3 week intervals from the beginning of May until 16 June during the two seasons. In each assessment, fruit having live larvae, symptoms of infestation or fruit that had dropped, but had webbing silk and/or faeces in their places were recorded. At each assessment date the trees and fruit were examined for any symptoms of crop injury. Statistical analysis was carried out based on the damaged fruit and the percentage reduction was based on Abbot's formula.

Cadra spp. Treatments

The same seven treatments were used to control *Cadra* spp.; however, the second spray took place 3 weeks after the first.

In 2006 and 2007, field trials were conducted in Dhakala Oasis to evaluate the seven spray regimes to control the almond moth, *Cadra* spp. Applications were done on 30 June 2006 and 21 July 2007. Evaluation of fruit infestation was made 15 September (during harvest) of each year. A motorized ground sprayer with a 600-L spray volume was used. All treatments were replicated three times. One date palm tree was considered as one replicate. Samples size was 100 fruit per one date palm taken at random from each replicate. Infested fruit dates were counted. Statistical analysis was done as above.

Meteorological conditions were recorded during the trials of both insects.

RESULTS AND DISCUSSION

Effect on *Batrachedra amydraula*

Tables 1 and 2 show the percentage reduction in *B. amydraula* infestation after fruit were treated by seven different regimes in 2006 and 2007. Following the second application, infestation levels across all treatments did not exceed 1% in 2006 and ranged from 1 to 2% in 2007. In the untreated replicates, the average infestation at the final assessment showed 13.5% and 18.6% in 2006 and 2007, respectively.

Tracer 240SC at 4.8 g ai/100 L (20 ml/100 L) was equal with Radiant 120SC at 1.8 g ai/100 L (15 ml/100 L) in both years of the study. Spinetoram was close to 2.7-fold more active than spinosad against this pest based on a grams active equivalence. Alternating spinosad with methoxyfenozide as a second spray showed similar efficacy regardless of the rate of methoxyfenozide (3.6-6.0 g ai/100 L). Tracer at 4.8 g ai/100 L as a first application followed by the lowest rate of Runner at 3.6 g ai/100 L (15 ml/100 L), showed the same efficacy as the maximum rate of Runner, especially in 2006.

Effect on *Cadra* spp.

Tables 3 and 4 show the percentage reduction of *Cadra* spp. infestation after fruit were treated by seven different control regimes in 2006 and 2007. In the untreated replicates, infestation reached 14 and 7.7% in 2006 and 2007, respectively. All the regimes showed the same efficacy in both years with infestation statistically equal and

ranging from 0.33 to 1.33%. Tracer 240SC at 4.8 g ai/100 L was equivalent with Radiant 120SC at 1.2 g ai/100 L when both were sprayed twice per season, indicating that spinetoram was 4-fold more active than spinosad on a gram active basis. A similar trend of results between the two spinosyns was indicated by Temerafuk (2007) when cotton leafworm was the target insect. This high level of control of *Cadra* in the field is important because if the infestation is not controlled in the field it will continue to damage fruit in the storehouses.

Meteorological Conditions

Tables 5 and 6 present the meteorological conditions during the trials for both insects. It appears that the activity of all products tested was not affected under these dry conditions and high temperatures reaching 51ºC. Also no crop injury was recorded. Temerak and Sayed (2001) also stated that Tracer was effective at the same temperatures without phytotoxicity.

CONCLUSIONS

One key pest management strategy is the rotation of different chemical modes of action to ensure that continuous selection for resistance does not occur and the possibility of pest resistance is avoided or at least significantly delayed (Thompson et al., 1997; Temerak, 2002). Application of a spinosyn insecticide in rotation with methoxyfenozide is an excellent example of a functional date palm integrated pest management program. Based on this strategy, it is recommended to use Tracer 240SC or Radiant 120SC at 20 ml/100L as the first spray, then Runner at 15 ml/100 L as the second spray to control *B. amydraula.* For the control of *Cadra* spp. Tracer 240SC at 20 ml or Radiant 120SC at 10 ml/100 L as the first spray and then Runner 240SC at 15 ml /100 L as the second spray can be advised.

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Tables

Table 1. Percentage reduction of *B. amydraula* infestation after fruit being treated with different rotation programs, New Valley, Egypt, 2006.

Treatment programs	Sampling date								Average	
	1 May		16 May		1 June		16 June			
	Infest. %	Red. %	Infest. %	Red. %	Infest. %	Red. %	Infest. %	Red. %	Infest. %	Red. %
1-Radiant/Radiant	5.9	58.7	0.6	96.5	0.6	94.2	1.0	91.8	2.0 *c*	85.1
2- Radiant/Radiant	3.8	73.4	1.8	89.5	0.5	95.1	0.2	98.3	1.5 *b*	88.8
3- Radiant/Radiant	2.8	80.4	0.6	96.5	0.2	98.0	0	100	0.9 *a*	93.3
4-Tracer/Tracer	4.4	69.2	1.4	91.8	0.4	96.1	0.0	100	1.5 *b*	88.8
5-Tracer/Runner	4.2	70.6	1.1	93.6	0.2	98.0	0.0	100	1.3 *ab*	90.3
6-Tracer/Runner	5.1	64.3	0.5	97.0	0.4	96.1	0.2	98.3	1.5 *b*	88.8
7-Tracer/Runner	3.3	76.9	0.2	98.8	0.0	100	0.9	92.6	1.1 *ab*	91.8
Control	14.3	-	17.2	-	10.4	-	12.2	-	13.5 *d*	-

LSD 0.05 = 0.49.
Figures followed by the same letter are not significantly different.

Table 2. Percentage reduction of *B. amydraula* infestation after fruit being treated with different rotation programs, New Valley, Egypt, 2007.

Treatment programs	Sampling date								Average	
	1 May		16 May		1 June		16 June			
	% Infest.	% Red.	% Infest.	% Red.	% Infest.	% Red.	% Infest.	% Red.	% Infest.	% Red.
1-Radiant/Radiant	6.4	53.2	0.2	99.1	1.0	94.5	1.3	92.8	2.2 *cd*	88.1
2- Radiant/Radiant	5.0	63.5	0	100	0.9	95.1	1.3	92.8	1.8 *bc*	90.3
3- Radiant/Radiant	3.3	75.9	0	100	0.3	98.3	0.8	95.5	1.1 *a*	94.0
4-Tracer/Tracer	5.7	58.3	0.2	99.1	0.2	98.9	1.6	91.1	1.9 *c*	89.7
5-Tracer/Runner	5.2	62.0	0.6	97.5	1.7	90.7	1.8	90.0	2.3 *cd*	87.6
6-Tracer/Runner	4.7	65.6	0.2	99.1	0.9	95.1	1.5	91.7	1.8 *bc*	90.3
7-Tracer/Runner	4.2	69.3	0.2	99.1	0.6	96.7	1.3	92.8	1.5 *b*	91.9
Control	13.7	-	24.2	-	18.4	-	18.1	-	18.6 e	-

LSD 0.05 = 0.36.
Figures followed by the same letter are not significantly different.
1. Radiant at 1.2 g ai/100 L applied twice at a 2 week interval.
2. Radiant at 1.8 g ai/100 L applied twice at a 2 week interval.
3. Radiant at 2.4 g ai/100 L applied twice at a 2 week interval.
4. Tracer at 4.8 g ai/100 L, applied twice at a 2 week interval.
5. Tracer at 4.8 g ai/100 L followed by Runner at 3.6 g ai/100 L applied at a 2 week interval.
6. Tracer at 4.8 g ai/100 L followed by Runner at 4.8 g ai/100 L applied at a 2 week interval.
7. Tracer at 4.8 g ai/100 L followed by Runner at 6.0 g ai/100 L applied at a 2 week interval.

Table 3. Percentage reduction of *Cadra* (*Ephestia*) spp. infestation at harvest after fruits being treated with different rotation programs, New Valley, Egypt, 2006.

Criteria	Programs							
	Radiant/ Radiant 1	Radiant/ Radiant 2	Radiant/ Radiant 3	Tracer/ Tracer 4	Tracer/ Runner 5	Tracer/ Runner 6	Tracer/ Runner 7	Control
% Infestation	0.66 *a*	1.0 *a*	0.33 *a*	1.33 a	0.66 *a*	0.66 *a*	0.33 *a*	14.0 *b*
% Reduction %	95.3	92.8	97.6	90.5	95.3	95.3	94.6	
LSD 0.05 = 1.48								

Figures followed by the same letter are not significantly different.

Table 4. Percentage reduction of *Cadra* (*Ephestia*) spp. infestation at harvest after fruits being treated with different rotation programs, New Valley, Egypt, 2007.

Criteria	Programs							
	Radiant/ Radiant 1	Radiant/ Radiant 2	Radiant/ Radiant 3	Tracer/ Tracer 4	Tracer/ Runner 5	Tracer/ Runner 6	Tracer/ Runner 7	Control
Infestation %	1.33 *a*	1.33 *a*	1.0 *a*	1.33 *a*	1.0 *a*	1.33 *a*	1.0 *a*	7.7 *b*
Reduction %	82.6	82.6	86.9	82.6	86.9	82.6	86.9	-
LSD 0.05 = 1.59								

Figures followed by the same letter are not significantly different.
1. Radiant at 1.2 g ai/100 L applied twice at a 3 week interval.
2. Radiant at 1.8 g ai/100 L applied twice at a 3 week interval.
3. Radiant at 2.4 g ai/100 L applied twice at a 3 week interval.
4. Tracer at 4.8 g ai/100 L, applied twice at a 3 week interval.
5. Tracer at 4.8 g ai/100 L followed by Runner at 3.6 g ai/100 L applied at a 3 week interval.
6. Tracer at 4.8 g ai/100 L followed by Runner at 4.8 g ai/100 L applied at a 3 week interval.
7. Tracer at 4.8 g ai/100 L followed by Runner at 6.0 g ai/100 L applied at a 3 week interval.

Table 5. Some metrological conditions during the trials of both insects (*B. amydraula* and *Cadra* spp.), New Valley, Egypt, 2006.

Criteria	*B. amydraula*			*Cadra* spp.		
	April	May	June	July	Aug.	Sept.
Maximum temperature (°C)	42.0	43.6	44	47	51.1	44
Average maximum temperature (°C)	32.6	38.4	39	41	42	40
Average minimum temperature (°C)	16.7	22.3	24.1	26	27.6	26.1
Average maximum relative humidity (%)	31.7	34.8	36	28	35	43.4
Average minimum relative humidity (%)	10	12	11	11	12	12

Altitude: 72 m; Longitude: 30°34' ; Latitude: 25°26'.

Table 6. Some metrological conditions during the trials of both insects (*B. amydraula* and *Cadra* spp.), New Valley, Egypt, 2007.

Criteria	*B. amydraula*			*Cadra* spp.		
	April	May	June	July	Aug.	Sept.
Maximum temperature (°C)	42.2	43.8	44	45.9	51.4	44.4
Average maximum temperature (°C)	32.8	38.5	39	41.4	42	39.8
Average minimum temperature (°C)	17	22.6	25	27.3	27.7	26.1
Average maximum relative humidity (%)	33	35	36	30	35	43.1
Average minimum relative humidity (%)	10	11	11	12	12	13

Altitude: 72 m ; Longitude: 30°34' ; Latitude: 25°26'.

Genetic Variability Analysis of Populations of *Fusarium oxysporum* f. sp. *albedinis*, Causal Agent of Bayoud Disease of Date Palm and Other *Fusarium oxysporum* Using Molecular Techniques

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Keywords: canary palm, RAPD, microsatellites ISSR, specific PCR

Abstract

The date palm (*Phoenix dactylifera* L.) is the biggest crop in Moroccan oasian ecosystem that produces dates and other products and preserves this system which is threatened by desertification. Several other constraints have also perturbed the development of the date palm sector, among them the Bayoud disease, caused by *Fusarium oxysporum* f.sp. *albedinis* (Foa) constitutes a serious threat for these oases. In order to control this disease, the use of resistant varieties was until now the most common way. However, the resistance durability depends on pathogen genetic variability notably the appearance of new physiological races. The last studies showed that the variability level was very low. Our research work aims to study the genetic variability in *Fusarium oxysporum* populations of 45 pathogenic and non pathogenic strains from different areas in Morocco and other Arab countries using specific and non specific PCR techniques. The pathogen strains of *F. o.* f. sp. *canariensis* (Foc) isolated from Canary island palm (*Phoenix canariensis* L.) have been used in this study. New RAPD and microsatellites ISSR primers were selected; these primers have generated 185 polymorphic markers. The dendrogram using average linkage and established by polymorphic bands revealed by RAPD and ISSR analyses showed the polymorphism in Foa strains without discriminating them to other strains and globally clustered the strains based on their geographic or isolation origins. The specific PCR using two specific couples of primers showed a relatively weak reliability level to detect Foa strains.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is the biggest crop in Moroccan oasian ecosystem that produces dates and other products and preserves this system which is threatened by desertification. The number of palm trees is about 4.7 million representing more than 230 cultivars (45%) and 2.7 million (55%) genotypes (natural hybrids) issued from seeds (Djerbi et al., 1986; Sedra, 1993, 1994, 1995, 2003b). Several other constraints have also perturbed the development of the date palm sector, of them the Bayoud disease, caused by the soilborne fungus *Fusarium oxysporum*. f.sp. *albedinis* (Foa) constitutes a serious threat for these oases. This disease is a part of plant diseases which are difficult to combat. The disease is located in the North African countries of Morocco, Algeria and discovered in some Mauritanian localities (Sedra, 1999, 2003a,c). Since it was first discovered in Morocco in 1870 (Malençon, 1950; Pereau-Leroy, 1958; Djerbi, 1982), it has destroyed approximately more than 13 million trees and several unique natural performant hybrids and celebrated commercial varieties have disappeared (Sedra, 2003a).

In order to control this disease, the use of resistant varieties was until now the most common way. Following the survey carried out in all Moroccan date palm plantations, 32 varieties were selected and field tested toward *F. o.* f. sp. *Albedinis*.

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Among these varieties, seven were found highly resistant (Louvet and Toutain, 1973; Louvet et al., 1970; Djerbi, 1988; Sedra, 1993a, 1995, 2003a). In Algeria, the celebrated resistant variety is 'Takerboucht' (Djerbi, 1988). However, among these selected varieties only 'Takerboucht', 'Boukhanni' and 'Sair Laylet' were of acceptable quality although certainly not equal to 'Deglet Noor' or 'Medjool', as famous commercial varieties. The evaluation of field resistance of six Iraqui varieties ('Barhri', 'Hallaoui', 'Khastaoui', 'Khadraoui', 'Saïr' and 'Zahdi') and six Tunisian varieties ('Boufeggous', 'Besser Lahlou', 'Gondi', 'Horra', 'Kenta', 'Kentichi') showed that none of them is resistant to Bayoud (Djerbi and Sedra, 1982; Sedra, 1992, 1995, 2003a). In Morocco, the recent finding showed the selection of many performant and resistant cultivars from the natural date palm population (natural hybrids) and from program breeding (Sedra, 1995, 2003a,b, 2005). Sixteen culivars, of which eight females and two males are promising, have been identified and characterized (Sedra, 2003b, 2005) according to descriptors determined by Sedra (2001). For examples of selected cultivars: female genotypes: 'Najda' (INRA-3014), 'Al-Amal' (INRA-1443), 'Al-Fayda' (INRA-1447), 'Boureehane' (INRA-1414) and 'Mabrouk' (INRA-1394) and male genotypes: 'Nebch-Bouskree' (INRA-NP3) 'Nebch-Boufeggous' (INRA-NP4). The studies of appreciation of the performance of this genetic material showed that these new cultivars present some agro-morphological characters more effective than those of the main Moroccan common varieties (Sedra, 2003b, 2005). Some among them like 'Najda', have already been multiplied in grand scale by tissue culture technique and distributed to farmers not only for farmers to reconstitute palm groves ravaged by the Bayoud disease but also to restructure the traditional palm groves that are less productive.

However, the resistance durability depends on pathogen genetic variability notably the appearance of new physiological races. The last studies using different molecular techniques showed that the variability level was very low (Tantaoui and Fernandez, 1993) and the pathogen isolates were cloned and have probably the same origin (Tantaoui et al., 1996). Moreover, an early detection method using the molecular markers is imperative for preventing further spread of the pathogen to uninfected date-growing regions. For this goal, the present research was started (Fernandez et al., 1998; Freeman and Maymon, 2000).

The main goal of this study was to evaluate the level of genetic variability in *Fusarium oxysporum* populations of various pathogenic and non-pathogenic strains from different areas in Morocco and other Arab countries using specific and non-specific PCR techniques. This research work also aims to determine whether the available molecular tools, RAPD and ISSR analysis and a species-specific primer pair for Foa, would facilitate reliable and accurate differentiation between the true date palm pathogen, and the related *F. o.*f.sp. *canariensis* species causing wilting of the Canary Island date palm.

MATERIALS AND METHODS

Fungal Cultures and Growth Conditions and Strains Origin

The 45 various strains of *Fusarium* used in this study (Table 1) were initiated from a single-spore culture and maintained on Czapeck growth medium at 25°C. The fungal cultures included pathogenic isolates from various date palm groves in Morocco (12 isolates), Algeria (6 isolates) and Mauritania (1 isolate), and other defined or undefined strains of *Fusarium* collected and issued from soil isolation using Komada medium (Komada, 1975), from date palm plantations in 12 Arab countries (22 isolates). Fungal isolates (KF2-SA and KF6-SA, FOS1-SU, FOS2-SU and FOS3-SU) of *Fusarium oxysporum* were received from the Agricultural Department respectively in Saudi Arabia and Sudan. Four isolates from Morocco of *F. o. çanariensis* were issued from infected leaves of the Canary Island date palm. For fungal DNA extraction, each strain spore suspension was inoculated into the surface of the liquid Czapek medium in the petri box. The fungal cultures were incubated under 25°C and continued lighting during 5-6 days. The mycelium carpet developed at the surface was recuperated under sterile conditions

and centrifuged at 6600 g during 5 min and dried between sterilised Wattman papers.

Fungal DNA Isolation, Purification and Quantification

The dried mycelium of each strain was lyophilized with a dry-freezer. Total DNA was extracted and purified as described by Lee and Taylor (1988) with some light modifications.

The RNA was eliminated by adding of proteinase k (RNase) (10 mg/ml). The DNA was dissolved in 200 µl TE buffer (10 mM Tris-HCl, pH 6.0, 1 mM EDTA; pH 8,0) and quantified and diluted to an approximate concentration of 5 ng/µl for PCR reactions.

PCR Amplification of Fungal DNA

The used primers were preselected among several primers because they permitted to reveal polymorphism on small DNA samples used for preliminary trials. The PCR techniques were optimized according to primers. For RAPD-PCR, 10-base oligomer primers used were: UBC-RAPD-269 (CCAGTTCGCC), UBC-RAPD-301 (CGGTGGCGAA), UBC-RAPD-381 (ATGAGTCCTG) and UBC-RAPD-391 (GCGAACCTCG), purchased from the University of British Columbia (UBC), (Vancouver, Canada). The reaction was achieved by one cycle 4 min of denaturation at 95°C followed by 30 cycles consisting of 1 min at 94°C, 1 min at 36°C and 1 min 30 s at 72°C. One cycle for 15 min at 72°C was conducted at the end. PCR reactions were performed in a total volume of 25 µl, containing 25 ng genomic DNA, 10X Taq buffer, 10 mM dNTP, 25 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (Promega) and 10 mM primer. For ISSR-PCR (Intern Simple Sequence Repeat), six selected primers used among 13 tested were: Mic6 ($(GATA)_4$), Mic43 ($(AGG)_6$), Mic48 ($(AG)_{10}$), Mic51 ($(TCC)_5$), Mic52 ($(ATG)_5AG$) and Mic54 ($(TCC)_5G$), purchased from Operon molecules for life (Cologne, Germany). The reaction was started by 1 cycle of 5 min at 94°C followed by 35 cycles consisting of 15 s at 94°C, 30 s at 51°C, 1 min 30 s at 72°C. One cycle for 7 min at 72°C was conducted at the end. PCR reactions were performed in a total volume of 25 µl, containing 25 ng genomic DNA, 10X Taq buffer, 10 mM dNTP, 25 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (Promega) and 10 mM primer

For Foa-specific amplification, PCR primers included two primer pairs; FOA1 (CAGTTTATTAGAAATGCCGCC) coupled with BIO3 (GGCGATCTTGATTGTATT GTGGTG), and FOA28 (ATCCCCGTAAAGCCCTGAAGC) coupled with TL3 (GGTC GTCCGCAGAGTATACCGGC) (Fernandez et al., 1998). Foa-specific PCR reactions were performed according to Fernandez et al. (1998) as follows: 1 cycle for 4 min at 95°C followed by 30 cycles for 30 s at 92°C, 30 s at 60°C and 30 s at 72°C for the FOA1-BIO3 primer pair; and 30 cycles for 30 s at 92°C, 30 s at 62°C and 45 s at 72°C for the FOA28-TL3 primer pair. Thereafter, a cycle of 15 min at 72°C was conducted. PCR reactions were performed in a total volume of 20 µl, containing 10-100 ng genomic DNA, 10X Taq buffer, 0.2 mM of dNTP, 1.5 mM $MgCl_2$, 1 unit *Taq* DNA polymerasc (Promega) and 1 µM primer, as previously described by Fernandez et al. (1998).

The PCR reactions were incubated in a TC3000 thermocycler (Progene, Techne England). All amplification products were separated in stained agarose gels (1.8% w/v; 15×10cm, W×L) with ethidium bromide in TAE buffer (Sambrook et al., 1989) electrophoresed with some modifications at 100 V for 1h 30 min. The DNA weight marker used was λ (lambda) digestive by enzymes Hind III and EcoR1. At the end of electrophoresis, the gels were visualized by UV illumination and photographed by Bioprint System 3000WL X-PRESS machine assisted by the computer (logiciel BIO-1D).

Data Analysis

Data were recorded as presence (1) or absence (0) of amplified products and the estimation of genetic distances between strain genotypes was analyzed by the logiciel SPSS and the Clustered dendrogram were constructed for 45 *Fusarium* strains.

RESULTS

Selection of Polymorphic Molecular Markers Using the RAPD and Microsatellites ISSR Primers

Four RAPD primers RAPD-269, RAPD-301, RAPD-381 and RAPD-391 and six ISSR primers (Mic6, Mic43, Mic48, Mic51, Mic52, Mic54) were selected because they have permitted to reveal interesting polymorphic DNA bands. All of the 65 RAPD DNA bands revealed were polymorphic and the total number of ISSR polymorphic bands reached 119 with 99.16% among total ISSR bands revealed (Table 3). Among 185 total RAPD and ISSR bands generated from 45 *Fusarium* strains, 184 were polymorphic (99.45%). In fact, the studied strains showed many different amplified DNA bands (Figs. 1 and 2), this seems that the variability level is interesting to analyse the *Fusarium* population.

Genetic Variability Analysis of the *Fusarium* Population Based on RAPD and ISSR Markers

1. RAPD Analysis. In general, for the RAPD profiles revealed by four primers, none of markers has permitted clearly to distinguish between all Foa strains and other strains. However, some Foa strains present near and similar profiles and none of the markers can distinguish the Foa strains according to their geographical origins. Figure 1 shows an example of profiles of strains Foa199-MA, Foa203-MA and Foa211-MA analyzed by primer RAPD-301. The used RAPD markers have thus revealed intra and interspecific polymorphism, and within the forma specialis (f. sp.) *albedinis*. The dendrogram (Fig. 3) realized on analysis basis of the treatment of matrixes constructed by polymorphic bands revealed by RAPD analysis of *Fusarium* strains, permitted to distinguish two groups: group I constituted by one strain Foa195-MA and the second grand group II is constituted by two sub-groups of which the first one (A) contains pathogen strain Foa80-MR from Mauritania and FOP1-SU from Sudan which was isolated from soil. The second sub-group contains the majority of the strains (B, C, D, E, F). The sub group B gathers pathogen strains from Morocco and Algeria. The sub group C contains the strains (KF2-SA, KF6-SA) from Saudi Arabia. Each subgroup D and E regroup only Moroccan pathogen strains while group F contains four Sudanese strains and one strain from Qatar. The genetic proximity between the strains is independent of their belonging or not to the (f. sp.) *albedinis*. Otherwise, this analysis permitted a subdivision based of the species, the specific forma and the geographical origin.

2. ISSR Analysis. The ISSR profiles of most *Fusarium* strains were remarkably similar, revealing characteristic bands for every primer. The six used primers have generated polymorphic bands. This polymorphism was noted within the pathogen strains of Foa and between the set of the studied strains, without discriminating the Foa strains of the other strains, but it permitted to distinguish globally the strains according to their geographical origin notably those of Saudi Arabia, of Sudan and also of the strains which have the same affinity as the strains of the *F. oxysporum* belonging the (f. sp.) *canariensis* (Fig. 2). Indeed, the dendrogram (Fig. 4) realized from the results of ISSR microsatellites primers permitted to classify studied strains according to their genetic distances in 2 groups. Group I is a group which united all strains Foc, three strains of *F. oxysporum* of different origins and one Foa strain from Morocco. Group II which collects the rest of the strains, is subdivided in two subgroups; the first subgroup divides on its turn in sub-subgroups containing the Foa strains and one *F. oxysporum* strain and one sub-group formed by only one Moroccan Foa strain; whereas the second sub-group is characterized by the fact that it regroups some strains from the same geographical origin.

3. RAPD and ISSR Analysis. From the results obtained by RAPD and ISSR techniques, 184 polymorphic markers have been used to construct a dendrogram (Fig. 5). This last regroups all *Fusarium* strains in only one group (group II) except an original Foa195-MA which is outside and constitutes alone the group I as it has been found previously by the two techniques separately. This Foa195-MA strain may be a particular strain. Group II is

subdivided in three sub-subgroups: the first one is very big; it regroups Foa strains from Morocco in a homogeneous group (A) and united separately the strains from Sudan with those of Qatar (B) finally one heterogeneous subgroup containing strains of *F. oxysporum* and of Foa from different origins. One sub-subgroup gathers two strains from Saudi Arabia and a strain from Oman that are separated by a relatively important genetic distance. Whereas the last sub-subgroup, it contains strains which have different origins and forma. The RAPD technique revealed higher polymorphism level than the one noted by ISSR technique. The microsatellites revealed similar profiles and bands for some strains, although these strains do not all belong to the (f. sp.) *albedinis* and do not have the same geographical origin. The combination of data of the two techniques permitted to ponder the big genetic divergence set in evidence by the RAPD technique between Foa strains.

Analysis of the Population of *F. o*. f. sp. *albedinis* and Other *Fusarium* Strains by the Specific PCR Technique

The amplification of genomic DNA by specific primer couple to Foa, TL3 - FOA28, revealed a specific band which has the size of 400 pb (Fig. 6) for 42.1% of strains belonging to the (f. sp.) *albedinis* (Table 3). In fact, the amplification of this band did not take place for 57.9% of Foa strains. However, this band has also been amplified in the case of six strains of *F. oxysporum* of which one is a (f. sp.) *canariensis* isolated from the Canary Island Palm. For amplification of a specific band of size 204 pb using the couple of BIO3-FOA1 primer, it was present for 63.2% of Foa strains (Table 3) and for seven strains which did not belong to the (f. sp.) *albedinis* or of which the belonging to this forma has not been confirmed. The assembly of the two types of bands revealed by two primer couples (TL3-FOA28 and BIO3-FOA1) only gave 30% of reliability (Table 3). The dendrogram based on recombined results obtained by two primer couples permitted to distinguish two big distinct groups (data not shown): the groups 1 and 2 are constituted by a mixture of Foa strains from different origins and *F. oxysporum* strains.

DISCUSSION

The genetic analysis of DNA of studied strains by RAPD and ISSR techniques revealed a polymorphism within the Foa analyzed strains. This result was due to presence of polymorphic bands. For some strains, obtained profiles are appreciably near and differ only by a restrictive number of bands. For others, profiles are completely different to those of the other Foa strains, and nearest even to some *F. oxysporum* strains originated from free Bayoud countries. The polymorphism noted in the studied Foa population not only exists between the strains which have different geographical origins (Moroccan, Algerian and Mauritanian), but within the strains from the same origins. Otherwise, the number of polymorphic locus revealed by RAPD primers is more important compared to those generated by ISSR primers of sequences microsatellites. The genetic diversity study has been done on a population of 45 strains including pathogen Foa strains from different origins, the *F. oxysporum* isolated from palm grove soils in Bayoud-free countries and contaminated ones and other strains bellowing to (f.sp.) *canariensis* (Foc). The used molecular techniques, based on PCR using the RAPD and microsatellites simple ISSR primers showed the polymorphism within Foa strains and also some *F. oxysporum* and Foc strains. This permitted to note that the used molecular markers have revealed high genetic variability level. It is very known that the *F. oxysporum* species is genetically very varied (Synder and Hansen, 1940; Booth, 1971; Kistler, 1997). Our results have detected a degree of variable polymorphism. The strains which did not reveal amplified bands, can be the strains whose variability has touched the hybridization site of primers used making them incompatible. If some strains possess few identical markers, other ones only differ by a restrictive number of markers. In fact, primers used for every molecular technique are efficient as polymorphism markers within the Foa strains. The comparable results have been reported by Sedra (2003a, 2006). The previous studies (Tantaoui and Fernandez, 1993; Fernandez and Tantaoui, 1994; Tantaoui et al., 1996) have concluded

weak diversity for Foa population. We think that this was due to the choice of used primers and number of markers used, number of sampled Foa strains and the strains for comparison. Moreover, the clustering of Foa strains in only one group of vegetative compatibility may be explained by the fact that this study only concerned a restrictive number of genes which could not permit to detect a variability within the Foa strains. While our recent molecular study proved this variability that was proved before when the aggressiveness level of Foa strains was appreciated (Sedra, 1993b,c). It was shown that Foa has generally some typical morphological and cultural characteristics (Sedra, 1993d), but recent findings showed that it may present variability in colony morphology (results non presented). On the other hand, the results of genetic analysis by the used markers revealed the proximity of the strains of Foa and other *F. oxysporum*. And in order to insure the belonging of *F. oxysporum* strains to the (f. sp.) *albedinis*, it is necessary to do tests of pathogenicity of the strains on a population of susceptible plants or other tests known as reliable markers (100%) that consider the variability and that are not unfortunately even available.

The proximity of genetic distance in this case can be explained by the level of probable evolution of the strains toward the (f. sp.) *albedinis*. We also noticed that the *F. oxysporum* strains from Bayoud-free countries are genetically near the Foa. We can say that these strains can be saprophytes or in evolution toward the pathogen (f. sp.) *albedinis*. The strains of Foa and these *F. oxysporum* can be derived from a common ancestor or the *F. oxysporum* that are an ancestor of which the Foa was derived. Indeed, according to this result, we cannot exclude the possibility of a mutation of the genes coding for virulence of these *F. oxysporum* strains that can make the forma special *albedinis* appear. RAPD markers revealed a better genetic divergence between Foa strains than ISSR microsatellite technique; because the RAPD technique targets arbitrary locus on the whole of the genome; this allowed the detection of a possible polymorphism. Besides, the used ISSR markers can serve to study the genetic proximity between Foa strains and some *F. oxysporum* saprophytes in order to determine the origin of Foa since they detected common monomorphic bands between some Foa and *F. oxysporum* strains. The regrouping of the matrixes of every studied marker confirmed the results of these two techniques and pondered the results of every type of analysis. This combination permitted to reveal a polymorphism between the Foa more moderate than the one obtained by RAPD markers and a proximity more than the one obtained by the ISSR microsatellite markers between some *F. oxysporum* and Foa strains.

The analyses achieved by specific PCR using the two couples of TL3-FOA28 bootjacks and BIO3-FOA1 showed a complementarity in their use; and that the second couple revealed the specific band of Foa more than the first. It lets think about the low level of reliability of this technique since it permitted to make the specific band for some *F. oxysporum* strains appear. The regrouping of Foa and the *F. oxysporum* strains in the same genetic group following the analysis, prove that the used molecular markers are not linked to the virulence markers. Therefore they cannot be used especially in the detection of the Foa when the variability had several origins, but rather in the study of genetic polymorphism within the (f. sp.) *albedinis*.

CONCLUSIONS

In conclusion, the study of genetic diversity of the strains based on molecular RAPD and ISSR microsatellites markers revealed a non negligible polymorphism on the one hand within the (f. sp.) *albedinis*, and on the other hand a genetic proximity between some Foa and *F. oxysporum* strains; this permits to formulate a new hypothesis on the origin of the Foa, that suggests that the parasite would derive from a saprophyte strain in all countries without needing to disseminate from the originated strain. It would be very useful to use other primers revealing a polymorphism between the Foa strains to give a more precise idea on the genetic structure of the population of this parasite. This would permit to foresee the possibility of a diversification of pathogenic faculty of the parasite and the possibility of the apparition of physiological races capable to surmount selected

resistances for the palm tree. Finally, obtaining of a better approximation of the extent of existing genetic variation, as covering a large part of the genome rests on the use of several polymorphic markers and the combination of found information and results. This returns the possibility to better know the genetic determinism of the pathogen virulence and to develop linked markers to genes of virulence.

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Tables

Table 1. *Fusarium* strains used in this study and their origins.

Fusarium species	Isolate	Origin	Host /soil
F. o. f. sp. *albedinis* (Foa)	Foa123-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa133-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa183-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa195-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa199-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa203-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa211-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa224-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa239-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa404-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa409-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa440-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa441-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa405-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	FOIKL-MA	Morocco	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa309-Al	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa602-AL	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa604-AL	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa605AL	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa606-AL	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa608-AL	Algeria	date palm leaf
F. o. f. sp. *albedinis* (Foa)	Foa80-MR	Mauritania	date palm leaf
F. oxysporum (Fo)	FO213-MA	Morocco	date palm root
F. oxysporum (Fo)	Foa234-MA	Morocco	date palm root
F. oxysporum (Fo)	FO281-MA	Morocco	date palm root
F. oxysporum (Fo)	FO552-MA	Morocco	date palm root
F. oxysporum (Fo)	FOTH2-MA	Morocco	soil
F. oxysporum (Fo)	FO127a-MA	Morocco	date palm root
F. oxysporum (Fo)	FOA1-EG	Egypt	soil
F. oxysporum (Fo)	FOC1-EG	Egypt	soil
F. oxysporum (Fo)	KF2-SA	Saoudi Arabia	date palm
F. oxysporum (Fo)	KF6-SA	Saoudi Arabia	date palm
F. oxysporum (Fo)	FOP1-SU	Sudan	soil
F. oxysporum (Fo)	FO08-SU	Sudan	soil
F. oxysporum (Fo)	FOS1-SU	Sudan	date palm
F. oxysporum (Fo)	FOS2-SU	Sudan	date palm
F. oxysporum (Fo)	FOS3-SU	Sudan	date palm
F. oxysporum (Fo)	FO2-OM	Sultanate of Oman	date palm leaf
F. oxysporum (Fo)	FOAP-MR	Mauritania	soil
F. oxysporum (Fo)	FOC1-QR	Qatar	soil
F. oxysporum (Fo)	FOP1-JO	Jordan	soil
F. o. f. sp. *canariensis* (Foc)	Foc882-MA	Morocco	date palm leaf
F. o. f. sp. *canariensis* (Foc)	Foc883-MA	Morocco	date palm leaf
F. o. f. sp. *canariensis* (Foc)	Foc884-MA	Morocco	date palm leaf
F. o. f. sp. *canariensis* (Foc)	Foc885-MA	Morocco	date palm leaf

These *Fusarium* isolates were isolated, purified and stored in sterilized sand in the laboratory of Dr Sedra M.H. (INRA, Marrakech, Morocco) since two to several years ago according to the isolates.

Table 2. Number of amplified and polymorphic bands generated by using selected RAPD and ISSR primers and percentage of polymorphic bands revealed on all studied *Fusarium* strains.

PCR technique	Primer	Number of amplified bands	Number of polymorphic bands	Percentage of polymorphic bands (%)
RAPD	RAPD-269	15	15	100
	RAPD-301	18	18	100
	RAPD-381	18	18	100
	RAPD-391	14	14	100
Total RAPD	-	65	65	100
ISSR Microsatellites	Mic 6	16	16	100
	Mic 43	23	22	95.65
	Mic 48	21	21	100
	Mic 51	22	22	100
	Mic 52	19	19	100
	Mic 54	19	19	100
Total ISSR Microsatellites	-	120	119	99.16
Total general	-	185	184	99.45

Table 3. Percentage of *Fusarium oxysporum* f. sp. *albedinis* (Foa) strains detected by specific PCR.

Primer couple used in specific PCR	Percentage of positive detection of Foa strains (% reliability)
TL3- FOA28	42,1
BIO3-FOA1	63,2
TL3- FOA28 / BIO3-FOA1	30

Figures

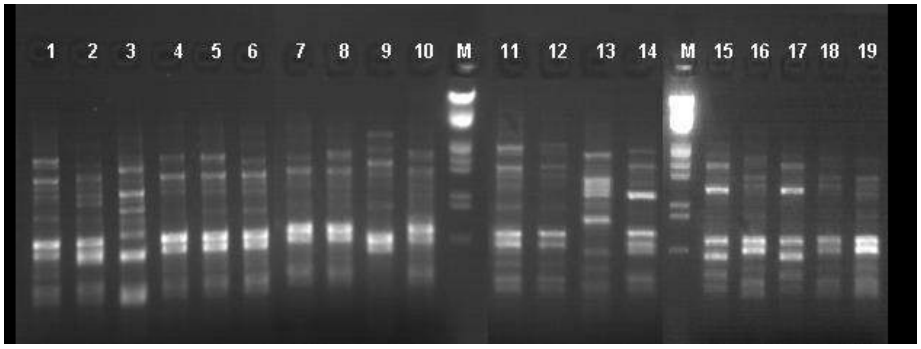

Fig. 1. RAPD-PCR of some genomic DNA of *Fusarium* isolates using primer RAPD-301. Electrophoretic profile on agarose gel 1.8% of PCR products. Lane M contains DNA markers with sizes in pb, λ/ Eco R1/Hind II/BAP, Pathogen strains *F.o.* f.sp.*albedinis* from Morocco (1 to 6, 11, 12, and 19): 1 :Foa133-MA ; 2 : Foa183-MA ; 3 : Foa195-MA ; 4 : Foa199-MA ; 5 : Foa203-MA ; 6 : Foa211-MA ; Pathogen strains from Algeria (7 to 10): 7 : Foa309-AL ; 8 :Foa602-Al ; 9 : Foa604-Al ; 10 : Foa605-AL ; 11 : Fo405-MA ; 12 : FOIKL-MA ; F.o. from soil (Egypt (13 and 14): 13 :FOA1-EG ; 14 :FOC1-EG) ; Pathogen strains of *F.o.* f.sp.*canariensis* from Canary Islands date palm, Morocco (15 to 18): 15: Foc882-MA ; 16 : Foc883-MA ; 17 : Foc884-MA ; 18 : Foc885-MA ; 19 :Foa123-MA.

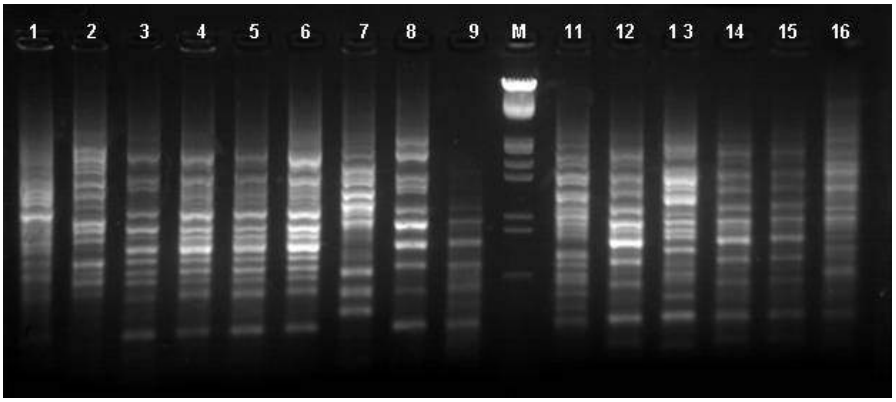

Fig. 2. Microsatellite ISSR-PCR of some genomic DNA of *Fusarium* isolates using primer Mic43. Electrophoretic profile on agarose gel 1.8% of PCR products. Lane M contains DNA markers with sizes in Kb, λ/ Eco R1/ Hind II/ BAP, Pathogen strains *F. o.* f. sp. *albedinis* from Morocco (1 to 3, 8, and 9): 1:Foa133-MA; 2: Foa183-MA; 3: Foa195-MA; Pathogen strains from Algeria (4 to 110): 4: Foa309-AL; 5: Foa602-Al; 6: Foa604-Al; 7: Foa605-AL; 8: Fo405-MA; 9: FOIKL-MA; *F. o.* from soil (Egypt (10 and 11): 10: FOA1-EG; 11: FOC1-EG; Pathogen strains of *F. o.* f. sp. *canariensis* from Canary Islands date palm, Morocco (12 to 15): 12: Foc882-MA; 13: Foc883-MA; 14: Foc884-MA; 15: Foc885-MA.

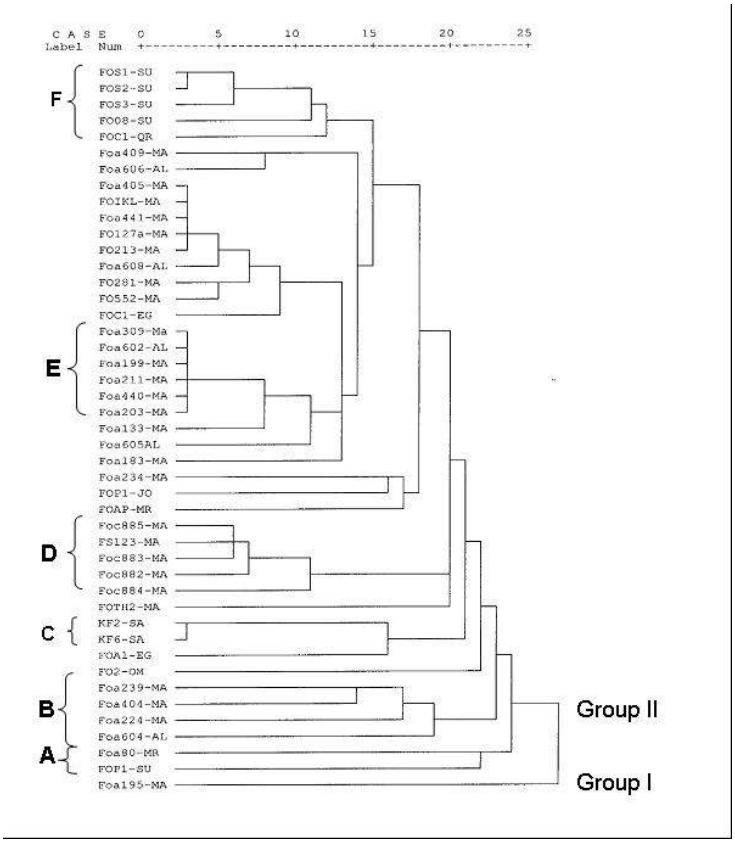

Fig. 3. Dendrogram of 45 *Fusarium* strains generated by group average clustering analysis using RAPD-bascd genetic distance.

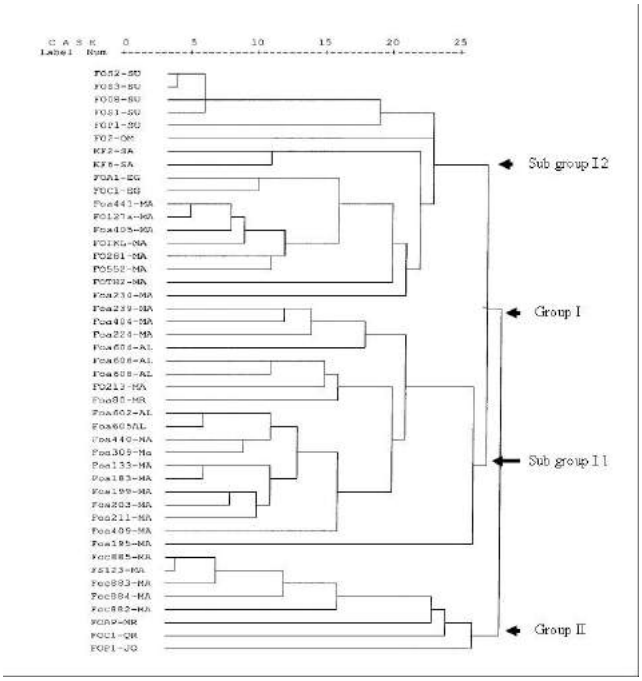

Fig. 4. Dendrogram of 45 *Fusarium* strains generated by group average clustering analysis using ISSR (microsatellite)-based genetic distance.

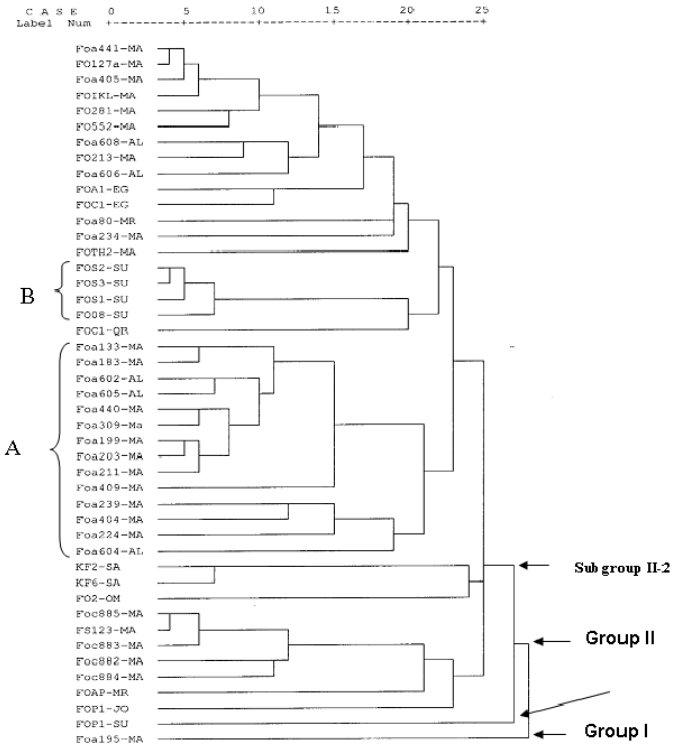

Fig. 5. Dendrogram of 45 *Fusarium* strains generated by group average clustering analysis using combined RAPD and ISSR (microsatellite)-based genetic distance.

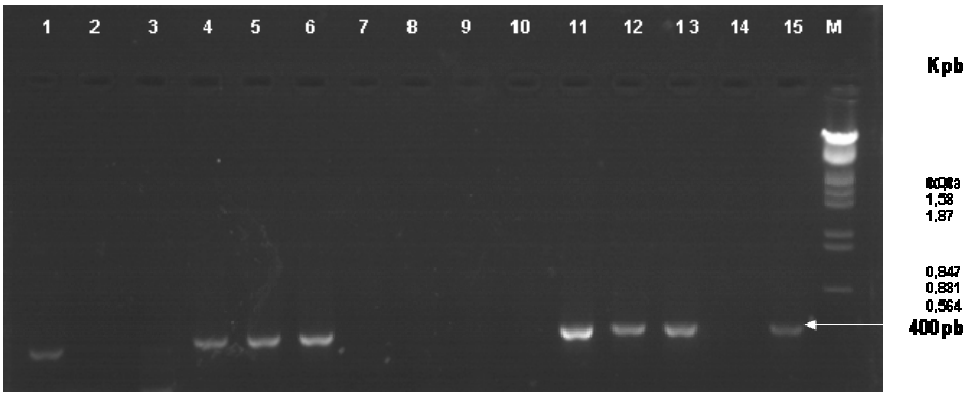

Fig. 6. Specific PCR Profil of some genomic DNA of *Fusarium* isolates using primers couple TL3-FOA28. Electrophoretic profile on agarose gel 1.8% of PCR products. Lane M contains DNA markers with sizes in Kb, λ/ Eco R1/ Hind II/ BAP Pathogen strains *F. o*. f. sp. *albedinis* from Morocco (1 to 11): 1:Foa133-MA; 2: Foa183-MA; 3: Foa195-MA; 4: Foa199-MA; 5: Foa203-MA; 6: Foa211-MA; 7: Foa224-MA; 8: Foa Foa239-MA; 9: Foa404-MA; 10:Foa409-Ma; 11:Foa440-Ma; Pathogen strains from Algeria (12 and 13): 12: Foa309-AL; 13:Foa602-Al;*F. o.* From Sudan: 14: FOP1-SU; Pathogen strains of *F. o*. f. sp.*canariensis* from Canary Islands date palm, Morocco: 15: Foc883-MA.

The Relationship between the Fungus *Fusarium solani* and Some Pathological Phenomena on Date Palm Trees and the Effectiveness of Some Systemic Fungicides for Their Control

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Keywords: palm deterioration, fronds yellowing, date numbness, mycotoxins, Iraq

Abstract

Many pathological phenomena are spread in date palm groves in Iraq. The severity of the diseases, susceptibility of the varieties and the symptoms vary. Some of them start with yellowing and the deterioration of palm fronds gradually and ending with death, other symptoms show complete drought of the tree (embalming). The phenomenon of date numbness is also one of the symptoms. Many studies have shown some isolates of the fungus *Fusarium solani* as the main cause of these symptoms, and this has been proven by pathogenicity test experiments in the laboratory and field. The symptoms were varied depending on the degree of virulence and vulnerability to the production of mycotoxins of isolates. The effectiveness of many of the systemic fungicides were tested to control these phenomena and control through soil dressing and foliar spray. Carbendazim and Iprodine were the most effective against the phenomenon of yellowing and death of the palm, while Chinosole and phosphorus acid showed highly effective against the phenomenon of date numbness. The results showed that the fungus *Fusarium solani* is one of the most important factors causing these pathological phenomena, which constitutes a real challenge for the palm groves and should be taken in consideration in any management practice.

INTRODUCTION

Many problems were reported facing the date palm groves in Iraq over the last two decades, and these have led to degradation. Most of them were attributed to the neglect of farmers and poor services due to lack of economic returns as well as the proliferation of many important pests like Humera, dubas, stems borers, pathological phenomena and others. Therefore the number of palm trees was decreasing gradually. Previously Iraq was one of the first countries in the number of palm trees in the world with about 30 million trees in the seventies of the last century (Baker, 1972; Abdul-Hussein, 1974), compared with 9.5 million trees at the present time (Ministry of Planning, 2005). Laville in 1966 reported that the fungus *Thielaviopsis paradoxa* (Desegn) Hohn was responsible for the deterioration of palm trees in Iraq. The fungus in question had caused the death of 500 'Zahdi' palms in Baghdad (Al-Hassan and Abbas, 1987). Al-Bahadli et al. (1989) noted a similar situation in central and southern regions of Iraq and they thought that the fungus mentioned plays a key role in the weakness and the deterioration of the palm. Abbas et al. clarify in several studies for the years 1990, 1991, 1996 and 1997 the accompaniment of *Chalaropsis radicicola* with deterioration and considered this responsible for the deterioration of access and the bending head and death, while Zuwein (1992) mentioned that it was produced from dry rot in the head.

The phenomenon of date numbness increased in recent years and has spread to many provinces infesting different varieties of which the most important are 'Khastawi',

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'Khadraowi' and 'Braim'. Symptoms begin to appear in the chamri stage and the following stages, by the contraction of dates. Dates in this situation cannot be marketed and therefore the disease is responsible for causing direct losses which may lead up to the loss of all stalks on the palm. There are no studies on the causes of the situation and the information emerging from a working group established for this purpose indicated that the phenomenon is caused by physiological factors. Therefore, the present experiment was initiated to explore the relationship between the fungus *Fusarium solani* and some pathogenic phenomena on date palm trees in Iraq.

MATERIALS AND METHODS

Survey of Pathological Phenomena

This study was conducted in thirteen provinces, they are Basra, Maysan, Dhi Qar, Diwaniyah, Samawah, Karbala, Babylon, Najaf, Wasit, Baghdad, Diyala, Salah Aldin and Anbar. The survey was conducted during the second half of the year 2005, included detailed information on the orchard, the location and number of palm trees, the details of the injured palms, stages of the infection status, age of palm trees, the level of service and the date of the last control against diseases in the orchard.

The survey was conducted with the assistance of 93 persons working in the plant protection sections in the departments of agriculture in the mentioned provinces with the help of farmers. The study included 816 orchards and 336,133 palm trees. Orchards were selected randomly within the provinces mentioned. Any other information regarding the status of orchard and other pests around the same trees were also included as a record. The information was collected from each province in separate tables and then identified and selected. The reality of orchards in Iraq was made in general.

Isolation and Identification of Pathogens

The etiology of cases of deterioration of the palm trees were isolated and diagnosed through the examination of samples from the infected parts of palm. Different sections were cultured on PDA media after surface sterilization using a sodium hiboclorate concentration 0.05%. Basic taxonomic keys used in the diagnosis were Booth (1977), Barnett and Hunter (1972), Ellis (1971), Gilman (1975), Toussoun and Nelson (1977) and Watanabe (2002).

Use of Fungicides in the Control

1. Fungicides to Control the Phenomenon of Yellowing and Death of Palm. Two experiments were carried out. In the first experiment a group of fungicides was used and in the second experiment three of the fungicides with the addition of foliar fertilizers were used, as shown below:

Treatment with Fungicides. Five systemic fungicides were used as shown in Table 1 in three ways. First was foliar spray on top of the palm (palm top) and cover shoots with a solution of the fungicides by 30 L per palm using a 400-L high pressure sprayer. The second method is irrigation with fungicides solution, by making a circular trench located one meter from the trunk depth of 10-15 cm, and the amount of fungicide solution added was100 L per palm, and the third way was to combine the two previous methods (spraying and irrigation) in the same quantities and rates used for each fungicide. Trees were treated in every way of these methods once and twice. The trial was conducted using a randomized complete block design (RCBD) with three replicates (each replicate was one palm tree) for each treatment, while the water alone was for the control treatment (comparison) with the addition of the fertilizer treatment once or twice as a control treatment with treatments that received the foliar fertilizers (Table 1).

Treatment with Fungicides and Adding the Foliar Fertilizer. A foliar fertilizer (Nebta.L: B 20 20 20) consisting of: MgO: 0.02, S: 0.26, Fe: 156 ppm, Cu: 60 ppm, Mn: 80 ppm, Zn: 55 ppm, Mo: 16 ppm and P_2O_5: 96 ppm was added. This was added by spraying on shoots at a rate of 1.5 g/L with 30 L of a solution of fertilizer per palm tree using a

sprayer of 400 L in capacity mounted on the tractor. After one hour of fungicides treatment (three of the fungicides mentioned in Table 1) that had proven effective in influencing pathogenic fungi in the laboratory, Benlate, Bavistin and Score were included in the use of the fertilizer test. Three means of application were used (spraying alone, irrigation alone and spraying and irrigation together) applied once and twice. A randomized complete block design (RCBD) with three replicates (replicate was one palm) was used for each treatment, while the control treatment included the use of fertilizer alone once and twice in addition to the use of water. The results of the study and the levels of effectiveness have been evaluated through the development of disease index to describe the case of yellowing as follows:

Class	Description of the situation
0	No symptoms
1	Yellowing of the fronds by 10%
2	Yellowing of the fronds by 30%
3	Yellowing of the fronds by 50%
4	Yellowing of the fronds by 50% and above
5	Dry fronds

2. Fungicides to Control the Phenomenon of Date Numbness. One of orchards had been selected in the province of Maysan. Most of the palm trees which were classified as 'Khadraowi' were bearing symptoms of the disease. Samples of soil, roots, stems and stalks for all the palm trees were taken before and after treatment. Five systemic fungicides (Table 2) were chosen for the treatment of palms with five replicates per treatment (a replicate was one palm). Fungi were isolated from all the replications of the components listed above. Fungicides were added in a trench around the tree at the same time a solution of fungicide was sprayed on shoots, and the treatment was repeated after 45 days of the first treatment. Observations were recorded during the ratab stage. The total number of the stalks on the palm, the stalks number bearing symptoms of numbness and the incidence percentage were calculated (Table 2).

The Impact of Mycotoxins of Some Isolates of *Fusarium solani*

Liquid Czapex Broth prepared based on the way of Alexopoulos and Beneke (1962) was inoculated in beakers of 500 cm^3 capacity. One cm in diameter discs were taken from the petri dishes from one of two-week-old isolates of the fungus *F. Solani* cultured on the PDA medium, wrapped in black nylon bags and left for 30 days with continuous shaking. Filtrate separation was drawn under vacuum through suppression of a Buchner filter containing the type of Mellipore, were utterly distributed in three beakers of 250 cm^3 capacity. Five date palm seedling of six months in age were placed in each container. The same number of flasks containing Czapex Broth filtrate without fungus with three beakers containing sterile distilled water were used for comparison. Observations and symptoms resulting from exposing the seedlings to the fungus filtrate were recorded.

RESULTS AND DISCUSSION

The Survey of Pathological Phenomena

1. The Distribution of Infection in the Provinces. The results of the study showed obvious variation of pathological phenomena presence in the orchards of the provinces (Table 3), reaching a maximum rate of 35.41% in the orchards of Karbala, followed by Dhi Qar 32.5%, Salah Al-Din 26.27% and Babylon 25.9%, while the lowest rate was 1.6% recorded in the province of Samawah. The overall rate of the proportion of pathological phenomena in the country was 8.56% (Table 1). Previous studies conducted by Ghali (2001) in the governorates of Babylon, Basra, Karbala, Baghdad, Najaf and Diwaniyah indicated the highest percentage of decline in palm groves was in Basra

(34.26%), followed by Diwaniyah (20.4%), and that might be attributed to the concentration of the researcher to declines caused by the fungus, *T. paradoxa* alone.

2. Distribution of Symptoms on the Infected Palm Trees. The total number of infected palm trees was 28768. Symptoms were distributed by 34.7% to frond distortion, 74.6% for dry fronds, 28.4% for the drought summit, 29.6% for the bending head and 42.4% for the other cases including inflorescence rot and insect injuries caused by borers, Humera, dubas, termites and other (Table 4). Results observed in the rule of the symptoms of dry fronds, which is on the rise in recent years for several reasons, notably the lack of control of the pathological phenomena and further spread of the responsible fungi, including *Chalara paradoxa* and species of the fungus *Fusarium*. The isolates of fungi were associated with the case of yellowing, several types of fungi were isolated and tested for their pathogenicity on 6-month-old seedlings. This indicated that the main factor for the case of yellowing was attributed to infected base palm with the fungus *Fusarium solani*, which works on the analysis of timber vessels and the production of a high percentage of alcohol. These symptoms can be identified by the work of cutting to a depth of 10-15 cm in the trunk (at a height of 1.5 meters). Other fungi such as *T. paradoxa* caused additional weakening of date palm trees.

Isolation and Identification of Pathogens

1. Fronds Distortion and the Curve of the Summit Phenomena. The results of isolates and the diagnose of the pathological agents of these phenomena showed repeating presence of the fungus *Chalara paradoxa*, a fungus that causes Majnona disease on the palm (Djerbi, 1983). Other reasearchers such as Laville (1966), Al-Hassan and Abbas (1987), Al-Bahadli et al. (1989) and Ghali (2001), stated that the fungus was often found as spots gradually turn to black on the palm fronds and their bases, the fungus moves into the heart of the palm through wounds, and despite the fact that the fungus is a secondary agent and non-significant (Djerbi, 1983), the influence is clear on the palm trees, which are showing weakness as a result of other fungal pathogens or by age or because of the presence of borers, especially stalk borer *Oryctes elegans* (Ghali, 2001), as well as upon the availability of appropriate environmental conditions.

2. Fronds Drought and Drought Summit Phenomena. The results showed the repeating and presence of fungi species on the roots and crown area of injured date palms. The most important species belong to the fungus *Fusarium*, including *F. oxysporum*, *F. solani* and *F. moniliforme*. The most frequent fungus was *F. solani*, as well as the presence of the fungi *Diplodia phoenicum* and *Gliocladium* sp. and some species of bacteria. This finding is consistent with the findings of Abbas et al. (1996), Sarhan (2001) and Mansoori and Kord (2006). The relationship of the fungus *F. solani* to these two phenomena was improved. Some isolates have high pathogenicity,which led to the killing of 6-month-old seedlings after 30-45 days of inoculation.

3. Date Numbness Phenomenon. Table 5 shows that the fungi *Fusarium solani* and *Fusarium moniliform* were the most frequent pathogenic fungi in the samples examined with the percentages of 56.6 and 36.6%, respectively. Some isolates of the fungus *F. solani* demonstrated high pathogenicity. The presence of *Fusarium* fungi on the roots were higher compared with those in soil, trunks and stalks samples. The saprophyte fungi were also present in most samples.

The results indicate that all fungicides tested had a significant effect on the disease, as shown in Table 6 the overall rate of the disease index was less with Bavistin (Carbendazim), then Rover, followed by Score, Beltanol and finally Benlate. Although a high effectiveness was known for Benlate to control the fungi tested, it failed to achieve good control and that might be due to the nature of the commercial product (Jordanian product), and that the results in the field do not match exactly with the results in the laboratory. Some fungicides have had a higher impact in the field compared with its impact in the laboratory as is the case with Rover and Score, the reason may be due to the use of recommended concentrations in the field.

The Effectiveness of Fungicides in the Field with Two Foliar Fertilizations

Results presented in Table 7 show that foliar fertilization with the three fungicides (Benlate, Bavistin and Score) applied once or twice did not significantly reduce the rate of the disease index in general, and the fungicides maintained the same order of the degree of impact on the overall rate of disease index. The reasons for that may be attributed to the foliar fertilization which was relatively ineffective to lower the rate of the disease index, and may be caused by the inability of the fertilizer to penetrate the surface of the leaves and the plant or the inability of the leaves in absorption due to the nature and the outer surface of leaves that are covered with a thick and dry chitinous membrane.

Fungicides to Control the Phenomenon of Date Numbness

Table 8 shows that the fungicide Beltanol was more effective than the others as the proportion of infected stalks was 4%, followed by the fungicide Phostrol by 9%, then Nando by 11%, Proplant by 20%, and Swift by 32%. However, Nando was less than Phostrol in the severity of the injury.

The results indicated the ability of mycotoxins of some *Fusarium solani* isolates to kill 6-month-old seedlings submerged in the fungus filtrate after only three days, and the cross-sections of stems of seedlings showed the ability of toxins to kill xylem vessels which turned brown in color.

CONCLUSIONS AND RECOMMENDATIONS

The observations obtained during the follow-up of the yellowing phenomenon and death of the palm trees in the provinces of Baghdad, Diyala, Karbala, revealed that the phenomenon of yellowing is prevalent and starts at the lower fronds and extends to the heart of the palm resulting in death of the tree, which is caused by the presence of *Fusarium* fungi species, especially *F. solani* as the fungus species have the ability to convert sugar to xylose that results from the decomposition of hemicellulose to ethyl alcohol. This can be observed from the odor from injured palms when making a cut in the trunk at a height of 1-1.5 m from the base, and this is in agreement with Nord and Mull (1966) and Gong et al. (1979) who indicated the possibility of the fungus to convert mentioned sugar into alcohol. As explained in serial sections from the base of the palm to its summit, the presence of infection in the crown area and moved upwards to the top to a distance of 1.5-2 m from the base of the palm, where there was a relatively strong smell of alcohol. The fungus *Chalara Paradoxa* injury occurs after the weakness of palm. Fungal infection caused by *Fusarium* and the presence of wounds and borers help to enter this fungus to the heart of the palm and affects the terminal bud and work on analyzing hemicellulose and often leads to curvature of the palm. In another study John et al. (1983) noted that the fungus secretes xylanase enzyme, which is important in the analysis of hemicellulose, and confirmed the existence of palm yellowing symptoms where there are no symptoms or signs of the fungus *Chalara paradoxa*. Also there are saprophyte fungi infections causing palm fronds weakness after spotting fungi, including *Alternaria alternata*, that can reside heavily on the injured palm. Fungi responsible for the case of numbness are species of *Fusarium* fungus and some of the most aggressive isolates of the fungus *F. solani* that infect the roots and base of the palm. Some isolates produce mycotoxins that are transmitted through the xylem vessels and lead to the killing, and isolates with medium aggressiveness lead to a fierce numbness. Stalks appear from the summit area, where there are divisions and rapid proliferation of cells, and appear to avoid influencing the early stages of the fruit, but then fruit begin to fade reaching the chamri stage. As a result of the impact of mycotoxins' access to the xylem vessels in the stalks are killed and thereby obstruct water and nutrient elements. The use of fungicides has led to improvement in the case of fruit and the fungicide Beltanol was the most effective on the disease and can be used as addition to the soil as well as spray on the shoots at the same time. *Fusarium* species pose a threat to the palm groves because of the resulting cases of yellowing and death as well as the effect on production (dates). The

phenomenon of numbness leads to the deterioration of the quality of dates and that because they become unsuitable for marketing. Therfore, there is a need for better management of date palm orchards and that would include the seeking for fungus-resistant varieties and multiplication by tissue culture, as well as study the effectiveness of fungal bio-pesticides (*Trichoderma* spp.) added to the soil, and to study the difference between the fungus *Fusarium* isolates to produce toxins which can be related to each disease symptom.

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Tables

Table 1. Fungicides used in the experiment and utilization rates.

Fungicides	Chemichal group	Rate of use
Benlate 50 WP(Benomyl)	Benzimidazole	2 g/L water
Bavistin (Carbendazim)	Benzimidazole	2 ml/L water
Score (Difenoconazole)	Azole	0.5 ml/L water
Beltanol (Chinosol)	Quinoline	2 ml/L water
Rover (Iprodione)	Dicaroximide	2 g/L water

Table 2. Fungicides used to combat the phenomenon of date numbness.

Commercial name	Common name	Rate of use
Beltanol 50 SL	Chinosole	1.5 ml/L water
Phostrol 53.6 SL	Phosphorus acid	2.5 ml/L water
Proplant 72.72 SL	Propamocarb- HCl	1.5 ml/L water
Swift 50 SC	Carbendazim	2 ml/L water
Nando 500 SC	Fluazinam	1 ml/L water

Table 3. The distribution of the incidence of pathological phenomena in the orchards. Surveyed

Provinces	No. of orchards	No. of date palms	No. of infected palms	% of infection
Basra	20	5373	320	6
Maysan	227	84525	6390	7.56
Dhi QAR	135	21307	6924	32.5
Samawah	62	27295	437	1.6
Diwaniya	61	42265	3894	9.21
Najaf	10	1580	183	11.6
Karbala	21	10655	377	35.41
Wasit	120	63452	3492	5.5
Babylon	14	4171	1080	25.9
Diyala	79	18912	490	2.6
Baghdad	40	50545	1027	2.09
Anbar	9	3605	115	3.91
Salah Al-DIN	18	2499	643	26.27
Total	816	336133	28768	8.56

Table 4. The distribution of palm trees in the orchards, depending on the nature and stage of infection.

Provinces	No. of infected palms	Nature and stage of infection*				
		Frond deformation	Frond drought	Summit drought	Bending head	other**
Basra	320	109	214	30	260	237
Maysan	6390	3904	5615	3345	3654	1790
Dhi QAR	6924	1224	5708	88	860	5286
Samawah	437	139	206	116	168	20
Diwaniya	3894	2119	3589	1702	858	1335
Najaf	183	-	183	90	-	150
Karbala	3773	730	2893	220	490	2163
Wasit	3492	445	754	1937	1609	202
Babylon	1080	73	816	114	70	396
Diyala	490	29	203	249	244	59
Baghdad	1027	770	651	270	316	380
Anbar	115	115	115	-	-	-
Salah Al-Din	643	337	511	21	-	170
Total	28768	9991	21458	8182	8529	12208
%	100	347	74.6	28.4	29.6	42.4

* May contain some trees that have more than one infection.
** Inflorescences rot and insects injuries like borers, dubas bug and Humera.

Table 5. Repetition of fungi in the treatment before adding the fungicides.

The fungi	Soil*		Roots		Trunks	
	Repetition**	%	Repetition**	%	Repetition**	%
Fusarium solani	1	3.3	17	56.6	-	-
Fusarium moniliforme	-	-	11	36.6	-	-
Pythium aphanidermatum	-	-	1	3.3	-	-
Aspergillus niger	26	86.6	20	66.6	27	90.0
Aspergillus flavus	28	93.3	15	50.0	25	83.3
Aspergillus terries	17	56.6	9	30.0	-	-
Penicillium sp.	2	6.6	-	-	-	-
Rhizopus sp.	15	50	14	46.6	6	20.0
Mucor sp.	1	3.3	-	-	-	-
Chaetomium sp.	1	3.3	-	-	-	-
Cladosporium sp.	1	3.3	-	-	-	-

* Soil dilution 1/1000.
** Total repetition of fungus in six treatments of five replicates.

Table 6. Rate of the disease index of the five fungicides with fertilization for one time.

Variety	Treatment	Rate of the disease index*							Total	Mean
		Benlate	Bavistin	Score	Beltanol	Rover	Con.1	Con.2		
Braim	Spray	4	3.3	3.3	2.3	3.3	4	3.9	24.1	3.45
	Irrigate	2.7	3.7	4	3.3	3.3	4.8	3.8	25.6	3.66
	Spray+Irrigate	4	3	3.7	2.7	3.7	4	5	26.11	3.73
Berhi	Spray	3.7	3	4	4.3	4	5	4.5	28.56	4.08
	Irrigate	3.7	4.7	4	5	4.3	4.9	4.8	31.4	4.49
	Spray+Irrigate	4	3	4.3	4.7	4	4.3	3.9	28.2	4.03
Osta Omran	Spray	1.7	0.6	1	0.3	0.3	2.1	1.8	7.84	1.12
	Irrigate	2	0.6	0.6	1.3	0.7	2.3	1.9	9.4	1.35
	Spray+Irrigate	1.7	0.6	1	2.3	0.7	1.3	1.2	8.8	1.26
Zahdi	Spray	2.7	2.7	1.7	2.3	2.3	3	2.1	16.8	2.4
	Irrigate	2.3	1.3	2.3	2.3	2.3	2.8	2.9	16.2	2.32
	Spray+Irrigate	1.7	3	2	2.3	1.7	2.5	3	16.2	2.32
Total		34.2	29.5	31.5	33.1	30.6	41	38.8	238.7	2.85
Mean		2.85	2.46	2.66	2.76	2.55	3.42	3.24	19.95	2.85

* Three replicates for each treatment.

Table 7. Rate of the disease index of the five fungicides with fertilization for tow time.

Variety	Treatment	Rate of the disease index*								Total	``
		Benlate		Bavistin		Score		Cont1	Cont2		
		1FER*	2FER*	1FER	2FER	1FER	2FER				
Prem	Spray	4	2.7	3.3	2.7	3.3	3.3	3.8	2.5	25.6	3.2
	Irrigate	2.7	3	3.7	3	4	2.3	4	3.8	26.5	3.32
	Spray+Irrigate	4	4	3	3	3.7	3	4	4	28.7	3.59
Berhi	Spray	3.7	4	3	3	4	4.7	5	4.3	31.7	3.97
	Irrigate	3.7	4	4.7	4.3	4	4.3	4.9	4.6	34.5	4.32
	Spray+Irrigate	4	4.7	3	2.7	4.3	4.7	4.8	4.2	32.4	4.05
Osta Omran	Spray	1.7	1	0.6	1	1	1	2.1	1.9	10.3	1.29
	Irrigate	2	1.7	0.6	0.7	0.6	0.6	1.5	1.3	9	1.13
	Spray+Irrigate	1.7	1.7	0.6	1	1	0.7	1.3	1.1	9.1	1.14
Zahdi	Spray	2.7	2.3	2.7	2.7	1.7	2.3	3.1	2.9	20.4	2.55
	Irrigate	2.3	2.3	1.3	2.7	2.3	2.3	2.8	2.5	18.5	2.32
	Spray+Irrigate	1.7	2	3	3	2	2	3	3	19.7	2.47
Total		34.2	33.4	29.5	29.8	31.5	31.2	40.3	36.1	266	2.77
Mean		2.85	2.78	2.46	2.48	2.66	2.6	3.36	3.01	22.2	2.77

Three replicates for each treatment*

Table 8. The percentage of injury and severity of the infection after treatment.

Treatment	Stalks total number	Number of infected stalks	% of injury	Severity of the injury
Beltanol 50 SL	24	1	4	2% of stalks
Phostrol 53.6 SL	35	3	9	70% of stalks
Proplant 72.72 SL	26	5	20	70% of stalks
Swift 50 SC	41	13	32	1/80% of stalks 4/10% of stalks 8/2% of stalks
Nando 500 SC	27	3	11	5% of stalks
Control	35	35	100	Medium (30-70%) of stalks

Evaluation of Soil Receptivity of Date Palm Groves in Arab Countries to *Fusarium oxysporum* f. sp. *albedinis*, Causal Agent of Bayoud Disease of Date Palm

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Keywords: date palm, Bayoud disease, soil receptivity, *Fusarium*, wilt diseases, Arab world

Abstract

The Bayoud disease caused by *Fusarium oxysporum*. f. sp. *albedinis* of the date palm tree is one of the most dangerous diseases in the world which is difficult to control. It is now spread in some countries of North Africa and since its appearance, it has occasioned huge losses in Morocco and Algeria and it has been discovered in Mauritania in the last few years. The Bayoud disease constitutes a serious threat for neighbouring countries and other Arab and Islamic countries that produce dates. In the frame of a regional project on Bayoud disease of date palm executed by the AOAD in 15 Arab countries, this research aims to evaluate the level of soil receptivity in 12 countries represented by 40 date palm groves localities to the pathogen fungus. The results have permitted to develop simple techniques to produce fungus chlamydospores and to evaluate soil receptivity to the fungus by measuring the spore germination percentage using soil and soil extract during only 48 hours. The results showed significant differences in soil receptivity to the pathogen fungus according to the countries and regions in each country. Moreover, the results showed the same level of soil receptivity to several strains of the pathogen from different origins and presenting different pathogenicity levels. It was shown that nearly all Arab soils present high to middle level of receptivity to the fungus and some soils are important, for example soil of Al-Ghamr in Libya and some soils in Syria, Iraq and others found in other countries. Consequently, it is advised to take precautions to prevent the entry of the disease in the countries where soils showed high receptivity. This research gives an idea, not about the disease spread, but it permits to imagine the map of spread risk of the disease according to countries that are still free and threatened by the contamination. Also, it is possible to apply this technique to evaluate soil receptivity to other wilt diseases of vegetables and other crops.

INTRODUCTION

In the majority of Arab countries, the date palm tree (*Phoenix dactylifera* L.) produces dates and other products and preserves the system which is threatened by desertification. So, this tree plays social, economical and environmental roles. This tree adapted to difficult climate conditions and permitted to control desertification phenomena. Date palm cultivation is spread and concentrated in the Arab world where the palm tree number reaches more than 70 million trees which represent more than 70% of the palm world population (105 million). This important crop is attacked by a lot of pests (fungi, insects, birds, rats, etc) of which the incidence varies according to the countries and may reach to 35%. In addition to red date palm weevil, which causes huge losses in date palm groves in Arab and Asian countries and in Canary Island palm trees in Europe, the Bayoud disease, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. a*lbedinis*

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(Foa), is major destroying factor of date palm trees. Since its appearance in 1887 in Morocco according the literature (Malençon, 1950; Pereau-Leroy, 1958), it has occasioned huge losses in Morocco and Algeria and it has been discovered in Mauritania in the last few years (Sedra, 1999, 2003a,c). The qualitative and quantitative losses have been estimated to 13 million of destroyed palm trees and some thousands in Mauritania. This has conduced to the reduction of good quality date production and disappearance of several good varieties e.g., the cultivars 'Berni' and 'Idrar' (Pereau-Leroy, 1958) and many good quality natural hybrids (Sedra, 1995, 2003a). The Bayoud disease constitutes such a serious threat for these productive oases, neighbouring countries and other Arab and Islamic countries and other that produce dates. This wilt disease is a part of plant diseases which are difficult to combat. In order to control this disease, the use of resistant varieties was until now the most common way.

Research carried out in Morocco for several decades led to select new resistant varieties and cultivars (Louvet and Toutain, 1973; Saaidi et al., 1981; Sedra, 1994a, 1995, 2001, 2003a,b, 2005). Some of these new cultivars have been mass multiplied by tissue culture technique and diffused to farmers in order to reconstitute ravaged areas (Sedra, 2003a,b, 2005). However, some varieties of quality and high commercial value such as 'Mejhoul' remain the target of the devastating pathogen. Aiming at protecting these varieties, biological control could be a promising way. In this context, other researches carried out in Morocco allowed not only the discovery of suppressive soils (Sedra and Rouxel, 1989; Sedra et al., 1994a,b), but also the involvement of antagonistic soil microflora (fungi, bacteria and actinomyces) in the supressiveness (Sedra, 1993a, 2003a; Sedra and Maslouhy, 1994, 1995; Sedra et al., 1994a,b). Moreover, several of these microorganisms were isolated and showed an important in vitro antagonistic activity against the pathogen (Sedra and Maslouhy, 1994, 1995; Sedra et al., 1994b). Before releasing these microorganisms in open field, effective and safe methods allowing their establishment in the soil must be developed (Essariuoi and Sedra, 2008).

It is know that the soil can protect crops and trees against the pathogen. Rouxel et al. (1991) have reported many couples of soil-born fungi and crops. For date palm tree, several celebrated and commercial varieties tested, from contaminated or free countries, were shown to be susceptible toward the Bayoud disease (Sedra, 1992, 2003a). This important material may be exploited and valorised in suppressive or non-receptive soils. This receptivity may be evaluated by the effect of pathogen spore germination in soil (Sedra, 1985, 1993; Sedra et al., 1994a,b; Sedra and Bah, 1993). The previous results issued from this research have demonstrated significant difference in Moroccan date palm grove soils receptivity levels to the Bayoud disease and to the pathogen (Foa), but no information about other date palm grove soils in other Arab countries.

In the frame of a regional project on Bayoud disease of date palm executed by the AOAD in 15 Arab countries (2004-2008), this research work aimed to evaluate the level of soil receptivity in 12 member countries represented by 40 date palm groves localities to the pathogen fungus, by the evaluation of the soil effect on spore germination.

MATERIALS AND METHODS

Soils of Date Palm Groves in Arab Countries

80 soil samples were sampled from 12 countries representing 40 areas (two samples/area and three areas/country), except Egypt, (four areas and eight samples) and Mauritania (six areas and twelve samples). Table 1 and Figure 1 show the areas and countries. Two kg of each soil per sample and three samples per area were sampled in 2 m around the tree trunk and in two different depths: 0-20 and 20-60 cm. The soil samples were dried under ambient conditions in a laboratory designated place during three days and mixed. Then, all remnant plant and stones were eliminated. After, these samples were ground and sifted (diameter: down 200 μm) they were then wrapped and stored in cases made of paper. The quantity of 250 g per mixed sample representing each area was sent to Morocco for laboratory analysis.

Pathogen Chlamydospores Production

The study was based on the use of pathogen chlamydospores because they constitute the wide shape of soil storing in the case of the absence of palm host and other spreading material when the conditions are unfavourable. First, the inoculum was produced on PDA medium (Potato Dextrose Agar) during 15 days. The mycelium and conidia were washed by sterile distilled water and transferred into sterile thin sand of dunes of the Sahara. After 15 days in dark conditions, a lot of mycelial cells and conidia were transformed to chlamydospores (6 to 20 μm) which were often gathered in chains (Fig. 2). The contaminated sand was washed by sterile water which was concentrated by a centrifuge machine at speed 5000 g. The supernatant solution containing produced chalmydospores was rapidly moved by ultrasonic sound machine in order to free single spores. This operation was amplified by strong hand moving. The spore concentration was estimated by hematimeter cell and readjusted to 10^5 spores/ml.

Evaluation of Germination of Pathogen Chlamydospores

The soil samples were stored in dry conditions. In order to activate natural biological activity of the telluric microorganisms, the soil was humidified by sterile water up to 90% level and incubated in dark conditions under ambient laboratory temperature during two months.

Two techniques of germination trial were tested:

a) First technique: germination of chlamydospores in sterile distilled water (SDW) and in soil extract. The soil extract was obtained by adding 100 g of soil in 200 ml of SDW. This solution was mixed during for 30 min and then centrifuged in order to separate soil extract and solid soil. Two ml of SDW or soil extract were sampled and put in sterile tubes separately. Then two ml of chlamydospores solution were incorporated in each tube. Three tubes per treatment (SDW or soil extract) were used. Both tubes were incubated in dark conditions and 25°C during 36h. After the incubation time, a small sample of each tube was taken in and coloured by adding methyl blue in order to observe the spores under a microscope. The effect of soil extract on chlamydospores germination was assessed by the percentage of germination of 400 to 500 chlamydospores in each experiment.
b) Second technique: germination of chlamydospores directly in soil. The study of the germination of the chlamydospores of *F. o*. f. sp. *albedinis* placed in contact of soils was achieved using the technique inspired of the one described by Adams (1967) and applied by Rouxel (1978) for *F. o*. f. sp. *melonis*. Each soil sample was humidified by SDW at saturation level and put in sterile glass boxes. The chlamydospores were glued under pressure on circular tablets made by special paper (porosity: 0.45 μm). The tablets were placed into the soil in boxes which were incubated at dark conditions at 25°C during 36h. For each soil, four tablets were used. Then the tablets were slightly washed and coloured and observed under microscope. The effect of soil on chlamydospores germination was assessed as previously by the percentage of germination of 400 to 500 chlamydospores in each experiment.

For the first technique, the soil used was from Zagora in Draa Vallet in Morocco (D19-E), which is more receptive to the Bayoud disease (Sedra et al., 1994a). For the comparison of both soils, three Moroccan soils were used as controls: soil (D19-E), suppressive soil (M2-A) from Marrakech date palm grove and soil (E11-E) from Errachidia in Ziz Valley. The soil (M2-A) was reported as suppressive and had a negative effect on pathogen chlamydospores germination (Sedra and Bah, 1993). The pathogen strain Foa133-MA was used because of its high pathogenecity level on date palm plants (Sedra, 1993b, 1994b).

Evaluation of Effect of the Soil Depths on Germination of Pathogen Chlamydospores

For this experiment, the study was realized only on 12 samples (depth 0-20 cm) and 12 samples (depth 20-60 cm) from 12 countries. Table 2 indicates the countries and areas where the soils were sampled. The results were compared by using two techniques

reported below and the pathogen strain Foa133-MA.

Evaluation of Effect of Both Soils on Germination of Pathogen Chlamydospores

Based on similar results obtained in preliminary trials, this study was achieved on both soils (40 areas and 12 countries) using the second technique (soil extract) and directly in soils sampled in depth (20-60 cm) and the pathogen strain Foa 133-MA.

Evaluation of Behaviour of Different Pathogen Strains in the Soil

In order to compare the germination chlamydospores of different pathogen strains in soil, the study concerned 10 pathogen strains from Morocco, Algeria and Mauritania (Table 3). The germination of chlamydospores was assessed in SDW and in two Moroccan suppressive (M2-A) and receptive (D19-E) soils used as controls.

Data Analysis

The percentage of spores germination inhibition was calculated as:
Percentage of germination = A-B/A. A: value of spores germination in water and B: value of spores germination in soil or soil extract. The data analysis was based on the test of Newman and Keuls ($p=0.05$) that was used to compare means. The values which are followed by the same letter are not significantly different.

RESULTS

The global results have permitted to develop simple techniques to produce fungus chlamydospores and to evaluate soil receptivity to the fungus by measuring the spore germination percentage using soil and soil extract during only 36 hours.

Effect of Soil Depth on Pathogen Chlamydospores Germination

It was shown that the chlamydospores germination was statistically comparable in the case of both soil depths and compared techniques using SDW and extract soils (Table 2). These results have permitted us to use only the second technique of germination in soil extract and directly in soil depth (20-60 cm) for the trials followed. This choice was founded in that in this soil depth, the palm tree produces the important part of roots and the conditions of soil extract are nearest to natural conditions. However, significant differences have been observed between the effects of soils according to their origins (Table 2). In fact, the percentage of chlamydospores germination varies from 24.3 to 88.6%. The weak percentages were obtained in some soils, e.g., in Libya, Syria and Iraq and the suppressive soil control in Morocco. But, the higher percentages were shown in soils from Jordan, Sudan and Mauritania and the receptive soil control from Morocco.

Comparison of the Effect of Both Soils on Pathogen Chlamydospores Germination

Figure 3 showed that pathogen chlamydospores were not able to germinate in suppressive soil conditions e.g., soil of Marrakech (M2-A) in Morocco and if the spores can germinate, the germinated tubes of spores are very short and stop developing and decompose themselves. However, in receptive soil, these tubes are longer 5 to 10 times. Figure 4 shows that the percentage of inhibition of chlamydospores germination in soil was significantly different and varied from 1.8% in soil from the area of Al-Ghour kaded of Jordan to 93.3% in suppressive soil of Marrakech in Morocco. Some soils in Syria (area of Sabkha Al-Boum), Libya (Al-Ghamr), Iraq and Tunisia showed relatively higher percentages but these percentages were medium in Sudan (area of Abou Ranam), Qatar (Gharb Muntazah Al-Khour) and Egypt (Al-Dakhla) (Fig. 4). In other countries, these percentages were very weak. This seems that their telluric conditions are very favorable for pathogen spores germination.

Comparison of Behavior of Pathogen Strains toward the Soils

Table 3 shows that the behavior of 11 pathogen strains from different areas toward the soils was globally and statistically comparable for both strains and both trials. The

percentage of spore germination varies from 88.7 to 94.8% (average 91.6% in sterile distilled water (SDW) and from 75.0 to 91.8% (average 84.8%) in receptive soil and from 4.8 to 8.1% (average 6.5%) in suppressive soil (Table 3). These results confirmed that the pathogen Foa133-MA strain used in preliminary trials may represent both used strains.

DISCUSSION

The results showed significant differences in soil receptivity to the pathogen fungus according to the countries and regions in each country. In fact, the main results showed that the soils of date palm groves sampled in 40 areas from 12 Arab countries have a significant effect on chlamydospores germination of the pathogen, causal agent of the Bayoud disease. However, the majority of these soils showed high percentages of spores germination and some soils from Arab countries seem to be interesting because they may inhibit the chlamydospores germination, for example of soils: Al-Ghamr in Libya and some soils in Syria, Iraq and others found in other countries. It is important to pursue the research on these soils in the countries that are still disease free. This inhibition has been observed in soils which are very rich in microorganisms as bacteria, fungi and actinomyceta (results not shown). Previous findings showed that the inhibition of chlamydospores germination in soil has been related to soil suppressiveness to the Bayoud disease (Sedra, 1993a, 2008; Sedra and Bah, 1993; Sedra et al., 1994a,b). The antagonistic microorganisms isolated from suppressive soil were shown to be able to inhibit the spore germination, growth in soil and pathogenic activity of the pathogen on date palm plants, in comparison to non-antagonistic microorganisms and some fungicides effect (Sedra, 2008). Several authors showed that bacteria and *Fusarium oxysporum* saprophytes play a primordial role in soil suppressivenes to *Fusarium* wilt diseases (Tu et al., 1975; Rouxel et al., 1979; Scher and Baker, 1982; Lemanceau et al., 1988; Park et al., 1988; Tamietti and Pramotton, 1990; Sedra, 1993a; Sedra and Bah, 1993; Sedra and Maslouhy, 1994, 1995).

This present research showed also that the soil depth and origin of the pathogen strain do not have a negative influence on the results. In fact, the results showed the same level of soil receptivity to several strains of the pathogen from different origins and presenting different pathogenicity levels.

Consequently, it is advised to take precautions to prevent the entry of the disease in the countries where soils showed high receptivity. This research gives an idea, not only about the disease spread, but it permits to imagine the map of spread risk of the disease according to countries that are still free and threatened by the contamination. Also, it is possible to apply this technique to evaluate soil receptivity to other wilt diseases of vegetables and other crops. In fact, previous findings showed that suppressive or receptive soils present a comparable effect for several disease wilts of crops (Sedra and Rouxel, 1989). This simple and rapid technique achieved in 48h may permit to evaluate the receptivity level of soil to the pathogen.

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Tables

Table 1. Soils of date palm groves in Arab countries and areas.

Countries	Localities and areas	Countries	Localities and areas
Mauritania	Baghdadia (Tagant) Zribat (Tagant) Al-Wasita (Tagant) Kasr Al-Tarchane (Adrar) Tayert (Tagant) Atar (Tagant)	Jordan	Ghour Kabid Al-Ryane Al-Thouta Al-Janoubia
		Syria	Centre of Nakhil Tadmor Centre of Sabkha Al-Boum Dir Al-Zour (Al-Boukmal)
Morocco	Zagora (Draa Valley) Al-Errachidia (Ziz Valley) Marrakech (North of mountain of Atlas)	Iraq	Dyala Al-Rachidia Abou Tahin (Karbalaa) Al-Daoura (Baghdad)
Tunisia	Al-Hamma (South) Tozor (South) Nefta (South)	Qatar	Al-Sylia Oued Jalal Gharb Muntzah Al-Khour
Libya	Al-Jafra 1 Al-Jafra 2 Al-Ghamr	Sultanate of Oman	Al-Batna Al-Dakhilia Al-Charqia
Egypt	Al-Kharia Al-Dakhla Bir Ain Al-Tarfaia Frafra date groves	Yemen	Al-Drihmi-Al-Qadba (plain of Tohama) Ghir Bawzil (cost of Hadramawte) Al-Ghorfa wa Sayoun (Hadramawte Valley)
Sudan	Marwa Abou Ranane Al-Qareer		

Table 2. Effect of soil depth on chlamydospores germination observed directly in soil extract from chosen areas of date palm groves in studied countries.

Country	Locality	Percentage of chlamydospores germination in soil		Percentage of chlamydospores germination in soil extract	
		Depth 0-20 cm	Depth 20-60 cm	Depth 0-20 cm	Depth 20-60 cm
Syria	Center of date palm Tadmer	37.0 c	35.2 c	35.0 c	31.3 c
Iraq	Dyala	44.2 bc	42.2 bc	42.0 bc	38.6 c
Oman	Al-Batna	74.4 ab	75.8 ab	77.1 ab	75.6 ab
Egypt	Frafra oasis	68.1 b	66.2 b	60.8 b	60.7 b
Qatar	Jalal valley	78.1 ab	76.6 ab	78.6 ab	74.7 ab
Mauritania	Tayert	82.2 a	82.8 a	74.3 ab	76.8 ab
Tunisia	Tozor	73.5 ab	75.5 ab	72.2 ab	73.8 ab
Libya	Al-Ghamr	26.6 d	24.3 d	25.6 d	27.9 d
Sudan	Marwa	87.2 a	88.6 a	82.9 a	84.5 a
Jordan	Al-Ryan	82.3 a	81.8 a	81.7 a	82.9 a
Morocco	Zagora	79.8 ab	83.8 a	80.2 a	81.6 a

Average of percentage of chlamydospores germination observed directly in soil extract according to the soil depth. The values followed by the same letter are not significantly different (analysis test of Newman and Keuls for p=0.05).

Table 3. Percentage of pathogen chlamydospores germination into water and resistant and susceptible soils according to pathogen origins.

Pathogen strains	Origin of pathogen strains		Percentage of chlamydospores germination into		
	Country	Areas	Water (SDW)	Susceptible soil Zagora	Resistant soil Marrakech
Foa2-MA	Morocco	Draa valley	88.7 a	91.8 a	7.6 c
Foa133-MA		Draa valley	94.8 a	84.2 ab	6.5 c
Foa165-MA		Figuig	90.1 a	85.1 ab	5.2 c
Foa214-MA		Ziz valley	88.8 a	75.0 bc	4.8 c
Foa239-MA		Bani valley	94.2 a	83.2 ab	7.1 c
Foa413-MA		Saghro	93.5 a	82.5 ab	6.7 c
Foa309-AL	Algeria	Ghardaya	89.8 a	76.1 bc	5.8 c
Foa605-AL		Ghardaya	90.7 a	85.7 ab	6.4 c
Foa606-AL		Ghardaya	92.5 a	85.7 a	8.1 c
Foa80M-MR	Mauritania	Atar	93.2 a	84.2 ab	7.2 c
Average			91.6 a	84.8 ab	6.5 c

Average of percentage of chlamydospores germination observed directly in susceptible soil Zagora (D19-E), resistant soil of Marrakech (M2-A) and in water. The values followed by the same letter are not significantly different (analysis test of Newman and Keuls for p=0.05)

Figures

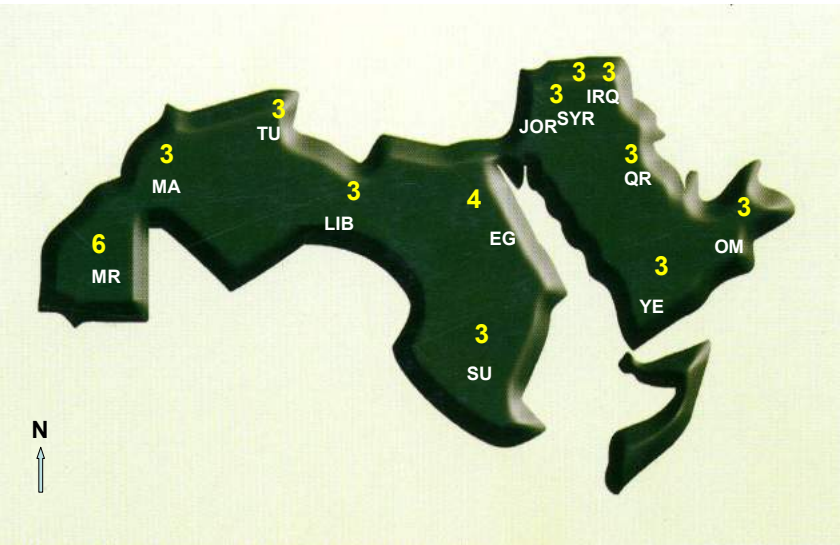

Fig. 1. Arab countries and number of areas (localities) where the date palm grove soils were sampled. MRT (Mauritania), MA (Morocco), TU (Tunisia), LIB (Libya), EG (Egypt), SU (Sudan), JO (Jordan), Syria (SYR), IRQ (Iraq), QR (Qatar), OM (Sultanate of Oman) and YE (Yemen).

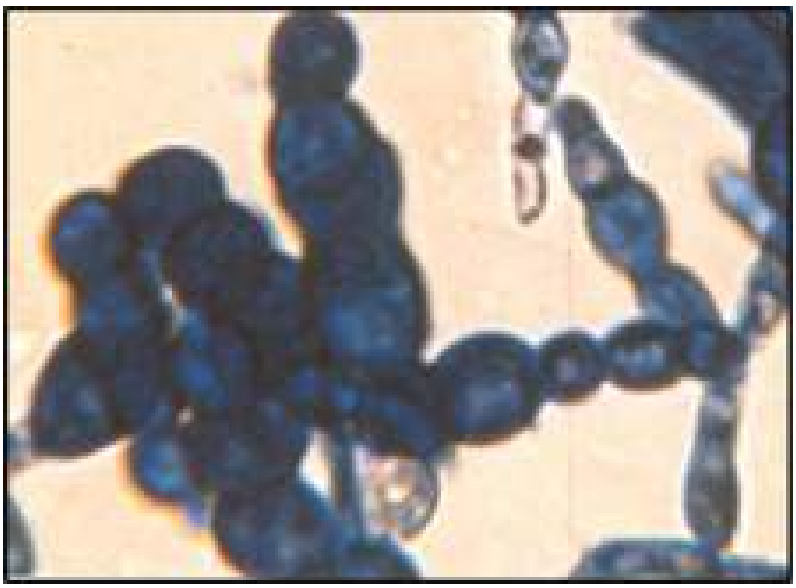

Fig. 2. Production of chlamydospores in chains of *Fusarium oxysporum f.* sp. *albedinis*, causal agent of the Bayoud disease of date palm tree.

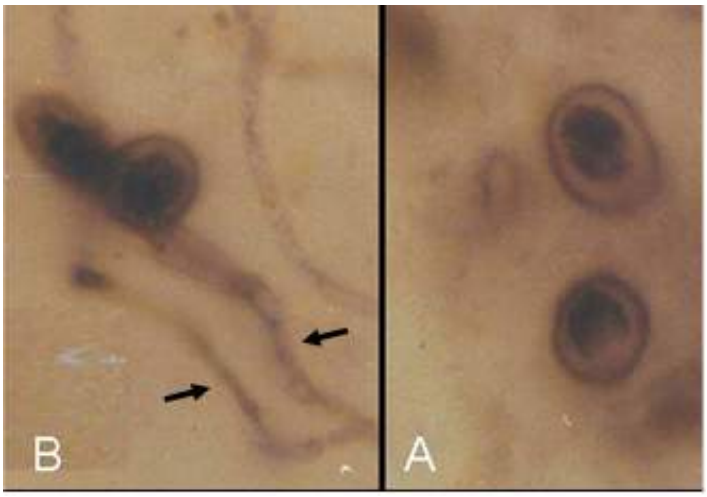

Fig. 3. Chlamydospores of *Fusarium oxysporum* f. sp. *albedinis*, causal agent of the Bayoud disease of date palm tree. A: Chlamydospores non germinated in suppressive soil. B: Chlamydospores germinated in receptive soil with long germinated tubes.

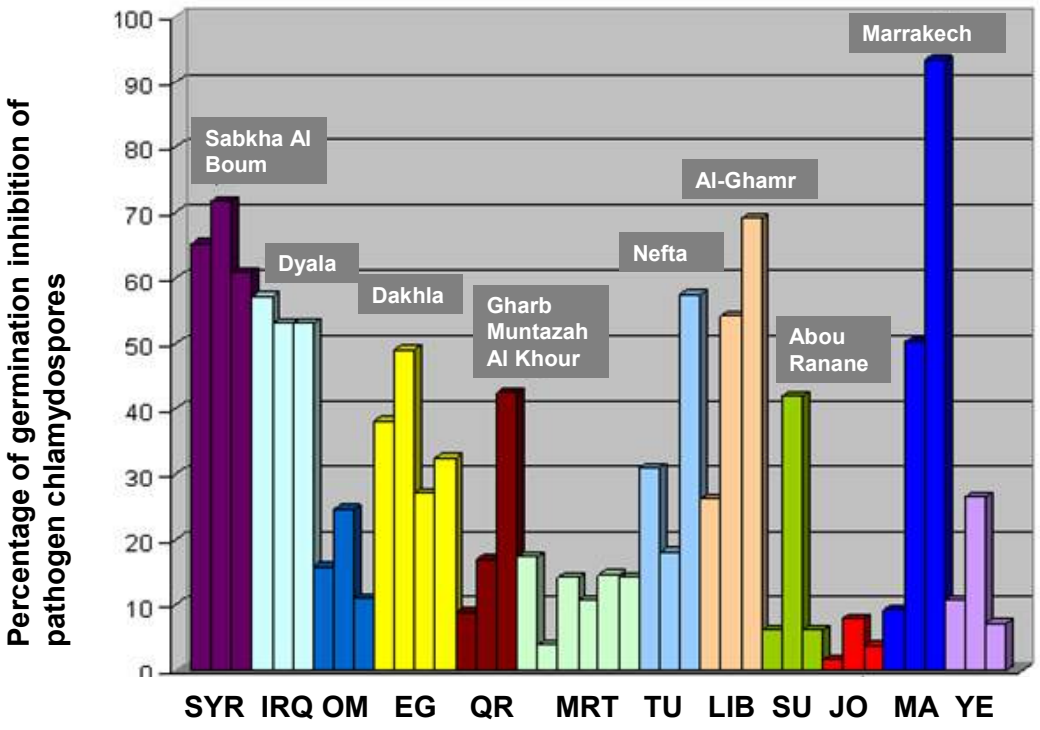

Fig. 4. Percentage of germination inhibition of pathogen chlamydospores of *Fusarium oxysporum f.* sp. *albedinis*, causal agent of the Bayoud disease of date palm tree in 40 date palm grove soils from 12 Arab countries. Syria (SYR), IRQ (Iraq), OM (Sultanate of Oman), EG (Egypt), QR (Qatar), MRT (Mauritania), TU (Tunisia), LIB (Libya), SU (Sudan), JO (Jordan), MA (Morocco) and YE (Yemen). The percentage values a: >80%, ab: 60-80%; b: 30-60%; c: 10-30%; c: <10% are significantly different (analysis test of Newman and Keuls for p=0.05). Some interesting soils are presented in the figure.

Ecopalm Ring Machine: Microwaves Technology for the Total Disinfestations of the Palm Trees Affected by the Red Palm Weevil

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Keywords: red palm weevil, *Rhynchophorus Ferrugineus* Olivier, *Phoenix Canarienis*, date palm infestation, date palm disinfestations, date palm treatments, plant treatment, ecological palm disinfestations

Abstract

The red palm weevil represents a serious threat for many countries where millions of date and coconut palms are cultivated. Millions of palms around the world are ruthlessly attacked and damaged by the *Rhynchophorus ferrugineus* Olivier, causing great economic loss to the growers. All the efforts to control this pest have not yielded the desired results. Most of the methods currently applied and recommended by experts for the preventive and curative palm treatments, are mainly based on pesticides and phytosanitary products use. The infestations control, using these products in urban environment and farms, is becoming more difficult, due to the limitations imposed by legislation on use of plant protection products, which are polluting and harmful to health. Therefore, the necessity and the need to seek more efficient and ecological alternative methods to address this serious emergency led our company, specialised for more than 20 years in high technology microwaves applications, to develop the patented Ecopalm Ring machine, based on the ecological microwave technology for the total disinfestations of the palms affected by the red palm weevil. Using the Ecopalm method has led to promising results in the fight against the red palm weevil by eradicating all the individuals hosted in the infested trunk, in whatever stage of their development they are found. The microwaves selectively destroy the eggs, larvae, pupae and adults, breaking and interrupting their life cycle completely, without causing any collateral damage to the palm treated, which is then saved. The Ecopalm method is very effective if used for preventive treatment of healthy palms, curative treatment of infected palms, and decontamination treatment of palms beyond recovery. An important aspect is the pure ecological method implemented, totally alternative to all methods known so far. No pesticides are used, no pollution is produced, and no harmful residues are left.

INTRODUCTION

The red palm weevil is a serious threat to many countries where millions of date and coconut palm trees are cultivated. All the efforts to control this pest have not yielded the desired results. Most of the methods currently applied and recommended by experts for the preventive and curative palm treatments are based on pesticides and phytosanitary products.

The Ecopalm Ring machine (Fig. 1) is a circular ring-shaped device and consists of two articulated modular sectors, to enable the ring to surround the palm trunk and to make the treatment in depth. The height of the device is 80 cm, the treatment covers a trunk portion of 1.05 metres, the internal diameter of the ring is 90 cm. The two articulated segments are equipped with electrical microwaves generators, called magnetrons, which generate high frequency electromagnetic waves necessary to eradicate the infestation.

The use of the Ecopalm Ring machine for the disinfestations of the palm trees from the red palm weevil is an efficient solution and an ecological alternative to all the

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methods actually used to control this dangerous infestation.

The objective of the work is to illustrate the promising results we have achieved in using Ecopalm technology, and to demonstrate the effectiveness of this method if used for preventive treatment of healthy palms, for curative treatment to recover infested palms, and for decontamination treatment of palms in advanced stage of infestation, beyond recovery.

MATERIALS AND METHODS

The machine is quickly placed around the infested part of the trunk, and started. The energy generated in high frequency electromagnetic waves, commonly named microwaves, travels quickly toward the centre of the palm trunk and penetrate in depth. The microwaves travel like in a fast track, across the air present in the cavities in a faster way than in the fibers of the plant, and go to strike directly all the individuals hosted there. The microwaves interact selectively with the water molecules that make up the organic materials of the pest. The overheating and hyperthermia generated by the microwaves vibrations ensures the mortality of all red palm weevil individuals present in the trunk, whatever the stage of their development is, like eggs, larva, pupae and adult, interrupting their life cycle completely and giving the possibility to recover the infested palm trees completely, if they are not found in an advanced stage of the infestation.

Four different testing treatments of four different palms from the species *Phoenix Canariensis*, have been effectuated. All trees were infested by the *Rhyncophorus Ferrugineus* Olivier (red palm weevil), and were found in different stages of the infestation.

The treatments consisted in using the Ecopalm Ring machine by surrounding the infested part of the trunk, and then in starting the application. The temperature, on the external layer of the trunks was maintained at 60°C for 30 min, which is the necessary time to devitalize the pest in this case.

The first two tests were done as curative treatments, and have been effectuated to demonstrate the effectiveness of the Ecopalm method to totally eradicate the red palm weevil infestation from the palm, to destroy the pest in whatever stage of its development was found, and above all, to demonstrate how the palm was totally recovered. During these two applications, the temperature in the two trunks was maintained at 60°C for 30 min, which is the time normally required by this method to devitalize the pest for this case.

The third test was effectuated to demonstrate the effectiveness of the Ecopalm method to decontaminate the palms trees in advanced stage of infestation and beyond recovery.

The last test was done in overtreatment to verify the endurance level of the palm, and to demonstrate the harmlessness of the microwaves for the plant tissue, even when the treatment time was quadrupled from 30 min necessary to eradicate the infestation to 120 min, and when the temperature on the external layer of the trunk was raised from the necessary 60 to 85°C.

All the palms used were subjected before the treatment, to husking, and cleaning. An infrared probe was placed near the infested part of the trunk, on the external layer to detect the temperature trend, during the treatment.

RESULTS

Curative Treatment for Palm A

Palm A was infested and attacked strongly in the central bud. It was treated maintaining the temperature of the external layer of the trunk at 60°C for 30 min with the Ecopalm Ring machine. The palm was left alive and subjected to periodic controls, every 20 days for a total of 90 days to assess the effects of the post-treatment intervention and to observe carefully the vegetative shooting, the development and the growth of its leaf apparatus. Three month later, in the spring time, the palm arrogantly pushed out new

preponderant buds, one year after the treatment, the palm was totally recovered (Fig. 2).

Curative Treatment for Palm B

Palm B was infested and attacked strongly in the central bud. The palm was treated maintaining the temperature of the external layer of the trunk at 60°C for 30 min with the Ecopalm Ring machine. Once the treatment was completed, the trunk was covered with several layers of polyethylene film, to maintain the new thermal state. After 48h from the end of the treatment, the trunk was cut into sections to verify the result of the disinfestations.

During the dissection of the trunk, 85 individuals from *Rhyncophorus ferrugineus* species (red palm weevil), were found in various developmental stages of their life cycle. All the individuals hosted in the trunk were found dead from which, 19 adults, 46 larvae, and 20 pupae (Fig. 3). The cavities created by the larvae, and by the exudates mounds have been affected by the microwaves in a greater extent than the surrounding vegetation which remained fresh, flourishing and hydrated. The microwaves travel like in a fast track, across the air present in the cavities in a faster way than in the fibers of the plant, and go to strike all the individuals hosted there. The internal layers of the treated trunk were heated in a smaller extent than the stages of the red palm weevil individuals, due to the different dielectric characteristics they have, and the lower amount of water molecules present in the vegetables than in the insect, consequently the palm tree did not suffer any damage.

Decontamination Treatment for Palm C

Palm C was in an advanced stage of infestation from the red palm weevil and beyond recovery. The palm was treated maintaining the temperature of the external layer of the trunk for 30 min at 60°C with the Ecopalm Ring machine. Once the treatment was completed, the trunk was covered with several layers of polyethylene film, to maintain the new thermal state. After 48h from the end of the treatment the trunk was cut into sections to verify the result of the disinfestations.

During the dissection of the trunk, 203 individuals from *Rhyncophorus ferrugineus* species (red palm weevil), were found in various developmental stages of their life cycle. The number of the individuals from the red palm weevil found dead was 198 from which 82 adults, 11 larvae, 10 pupae, and 95 individuals between pupae and adults in the cocoons (Fig. 4). 5 adults were found alive. It is to specify that the 5 adults alive have been found in a cavity, below the lower edge of the machine, and during the examination they were in an evident state of sufferance. These five individuals were kept under close observation for 24h. 2 individuals died within the first 12 hours, the remaining 3 within the following 24h. This final result led the mortality rate to 100%.

Overtreatment for Endurance Level Verification for Palm D

This testing was done in overtreatment to verify the endurance level of the palm D, and to demonstrate the harmless of the microwaves for the palm plant tissue, even when the treatment time was quadrupling from 30 min necessary to eradicate the infestation in this case to 120 min, and the trunk temperature was raised from the necessary 60 to 85°C. The palm was left alive to assess the effects of post-treatment intervention and to observe carefully the vegetative shooting of the plant, the development and the growth of the leaf apparatus.

The palm was subjected to periodic controls for 9 months. The leaves which have been overtreated for 120 min continued to grow regularly and to have a normal vegetation in the upper part of the treated area with the microwaves. A certain number of these leaves branches have been dissected, and it was noted immediately that beneath the external layer treated and slightly dehydrated, due to the oversized microwaves irradiation, the vegetal fibers remained flourishing and green, with a regular lymphatic flow (Fig. 5).

CONCLUSIONS

The results achieved with the Ecopalm microwave technology are promising. It is a new technology, for the first time applied on plants alive for disinfestations. It is an efficient ecological solution and an alternative to all the methods actually used to control the red palm weevil infestation. All the efforts to control this pest have not yielded the desired results yet. This method is ecological and clean; no harmful and toxic residues are left during and after use. It is safe for operators and for citizens. It can therefore be used in public and private places.

The Ecopalm method is efficient and will lead to promising results for the curative treatment of infested palms. The treatment eradicates the red palm weevil when the infestation is in progress and gives the possibility to save the palm. The method is easy and fast, in a few minutes the infestation is granted, and ensures the mortality of all the red palm weevils present in the trunk, in whatever stage of their development they are found, like eggs, larvae, pupae and adult, breaking and interrupting completely their life cycle. The palm does not suffer; the parts of the trunk and the branches treated with the microwaves remain fresh, flourishing and hydrated. The palm treated in time was recovered, pushed out arrogantly a new preponderant bud, and returned to be perfectly a normal and healthy tree, in less than one year .

The Ecopalm method is also efficient for the preventive treatment of healthy palms. After pruning the fronds, the branches that remain on the palm tree have a high rate of humidity, which encourages the diffusion of fungi and bacteria. These bacteria emit olfactory molecules that attract the red palm weevil. Palm trees are therefore particularly exposed to the attack. In natural conditions, it takes a palm tree several months to heal from pruning and to dry out the humidity caused by mould and bacteria. The use of the Ecopalm method, after cutting off the fronds, helps to eliminate this problem, and to sterilize the trunk surface from mould, bacteria and rot, in just five minutes.

The Ecopalm method is efficient for the decontamination treatment of palms before elimination found in advanced stage of infestation and beyond recovery. The use of the Ecopalm method in this case, could be an alternative to the methods actually used in many countries and consisting in cutting the trunk into many pieces and incinerating it. This operation is long and harmful, and is not efficient against the dispersal of the adult weevils.

The use of Ecopalm technology, with the implementation of the three methods of treatments above recommended, and following an accurate and well studied plan, helps in a first stage to slow down the advancement of the infestation, and contributes, in the second stage, to defeat it completely giving the great opportunity to rehabilitate entire infested areas.

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Figures

Fig. 1. The Ecopalm Ring machine is a circular ring-shaped device.

Palm A. Three months after the treatment. Palm A. One year after the treatment.

Fig. 2. Curative treatment of Palm A. Three months later, in the spring time, the palm arrogantly pushed out new preponderant buds. One year after the treatment, the palm was totally recovered.

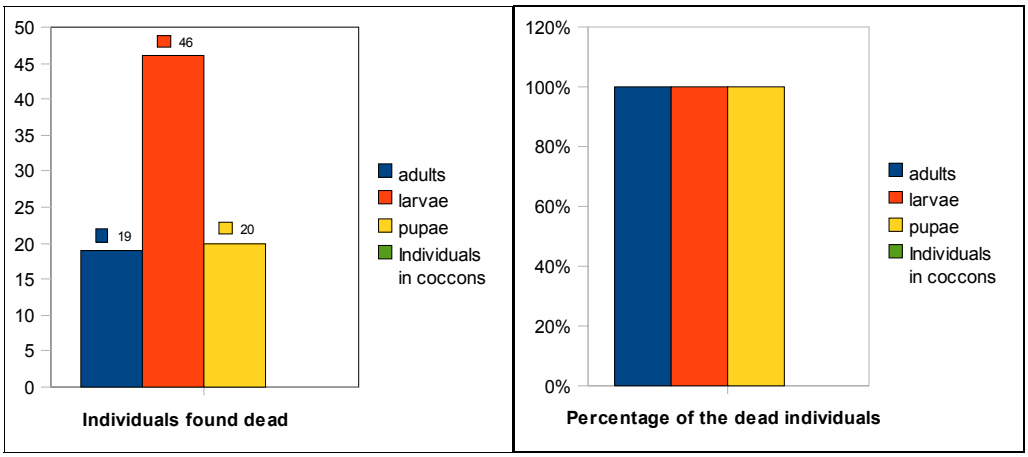

Fig. 3. Curative treatment of Palm B. Results of the inspection 48h after the treatment. The total individuals from the four stages found dead, were 85 from a total of 85.

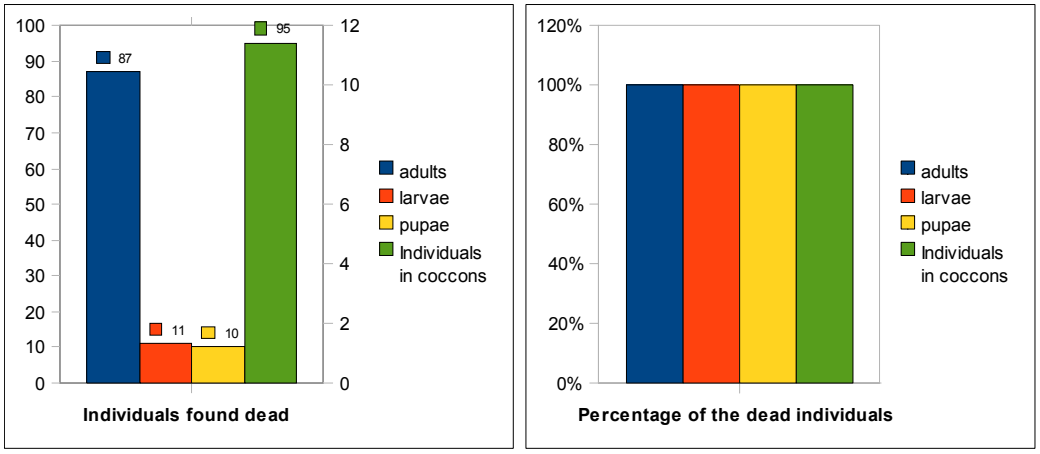

Fig. 4. Decontamination treatment of Palm C. Results of the inspection 72h after the treatment. The total individuals from the four stages found dead were 203 from a total of 203.

Regular lymphatic flow, leaves grow regularly, vegetation around cavities remains green.

Fig. 5. Overtreament for endurance level of “Palm D”.Beneath the external layer treated and slightly dehydrated due to the oversized microwaves irradiation, the vegetal fibbers remained flourishing and green, with a regular lymphatic flow.

The Montreal Protocol and the Methyl Bromide Phase Out in the Dates Sector

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Keywords: Montreal Protocol, ozone layer, methyl bromide, dates, critical use exemptions

Abstract

The meeting of the parties (MOP) to the Montreal Protocol called for the Methyl Bromide (MB) phase-out in 1992. In 1997, a global phase-out schedule of this chemical was established by the MOP: Article 5 countries are required to freeze consumption and production of MB by 2002, reduce its use by 20% in 2005 and complete total phase-out by 2015. Non-Article 5 countries had to phase-out MB by 2005. However, non-article 5 countries were allowed by the parties to present Critical Use Nominations (CUNs). The exemption application process is extremely rigorous. After analysis of the CUNs, MBTOC makes recommendations on the applications to the Parties to the Protocol. The Parties then determine whether or not to approve each application. The two countries presenting CUNs every year for dates' disinfestation are the US (California) and Israel. Consumption of MB for controlled uses has significantly decreased both in developed and developing countries. In A5 countries, phase-out has been achieved to a large extent through investment projects funded by the Multilateral Fund of the Montreal Protocol through its implementing agencies and some bilateral agreements. UNIDO designed a project proposal to address the issue of alternatives to MB for the palm date sector.

The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available in Algeria and Tunisia. UNEP and UNEP/ROWA have organised many activities in North Africa and in the Middle East to discuss and implement alternatives to MB for dates' disinfestation.

INTRODUCTION

Stratospheric ozone protects life on earth from the damaging effects of ultraviolet B radiation. In 1974, Molina and Rowland, from the University of California in the United States, proposed the hypothesis that stratospheric ozone was being broken down by volatile man-made Ozone Depleting Substances (ODS). This prediction was of enormous environmental importance and earned both of them a Nobel Prize in chemistry in 1995. In response to the resulting ozone hole being observed in the 1980s, the Vienna Convention to protect the ozone layer was set up in 1985 and in 1987, the Montreal Protocol (MP) was formed and this commenced regulation of ozone depleting gases.

Increased UV radiation on earth created by the ozone hole has many effects on organisms and human health. It affects plants and animals alike. Phytoplankton populations are reduced and this affects the food chain. In humans, increased exposure to UV-B increases the risk of skin cancer, cataracts and a suppressed immune system (Fahey, 2006). For example, in Australia it adds to their already high incidence of skin cancer, which is the worst in the world, with two out of three Australians being affected by it at some stage of their lives.

MB was included as an ozone depleting substance under the Copenhagen Amendment of the MP in 1992. Although MB has a much shorter half-life than many

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other ODS substances in the atmosphere, bromines are up to 50 times more destructive than chlorines and regulation of MB was considered to have immediate benefits to ozone layer recovery. The Copenhagen amendment set different phase-out schedules of 2005 and 2015 for industrialized (Non-A5) and developing (A5) countries, respectively. Quarantine and Preshipment (QPS) uses are presently exempted from controls, although consideration is being given to further regulation. Although many sectors in industrialized countries met the phase out of 2005, a large number of sectors sought to retain MB under the 'Critical Use' provisions of the Montreal Protocol, which allowed for continued use of MB if no technical or economical alternatives existed (Porter et al., 2009). Developing countries are expected to phase out by 2015 with a provision for 'Critical Uses' if required. The two non-Article 5 countries presenting CUNs every year for dates' disinfestation are the US (California) and Israel.

The objective of this paper is to present the trends in global MB consumption during the last 20 years, the decision IX/6, the CUNs in the dates sector presented by Israel and the USA, The UNIDO project for Algeria and Tunisia and finally, UNEP activities in North Africa and in the Middle East in the dates sector.

TRENDS IN GLOBAL METHYL BROMIDE CONSUMPTION

For the past six decades, MB has been the fumigant of choice to ensure effective soil disinfestation for preplant soil treatment in many horticultural sectors. The main reasons for this are its broad spectrum of activity, its high vapor pressure which allows it to act in the gaseous phase in the soil - a characteristic facilitating its distribution through the soil profile - its cost-effectiveness, and the comparatively short plant-back intervals necessary after application. Targets have traditionally included soil borne fungi, bacteria, viruses, insects, nematodes and weeds (Martin, 2003; MBTOC, 2007; TEAP, 2009).

Presently, over 90% of the remaining non-Quarantine and Preshipment (non-QPS) uses of MB (controlled uses) are for soil fumigation, and the remainder is for non-QPS treatment of commodities (rice, dates, chesnuts, pasta, cured pork) and structures (mills). The major crops using MB as a soil fumigant worldwide are tomatoes, strawberry fruit, peppers, eggplant, cucurbits, ornamentals (cut flowers and bulbs), orchards (for replant disease) and nurseries (including, strawberry runners) (TEAP, 2009).

Consumption of MB for controlled uses has significantly decreased since the MP and its Copenhagen Amendment entered into force, both in developed and developing countries. Figure 1 shows trends in MB consumption in Non-A5 and A5 countries for the period between 1991 and 2007. The global consumption of MB for controlled uses was estimated to be about 64,420 metric tons in 1991 and remained above 60,000 tones until 1998 (TEAP, 2009).

The official baseline (1991 consumption) for Non-A5 countries was 56,043 tons in 1991 and since then, consumption has declined to 6,966 tons in 2008 or 12% of the baseline. The official baseline for A5 countries (average consumption for the period 1995-1998) was 15,703 tons, rising to a peak consumption of 18,125 tons in 1998, declining to 6,226 tons or 40% of the baseline in 2007 (Fig. 1).

As mentioned above, MB consumption for controlled uses (non-QPS) in industrialized countries is only presently permitted under the Critical Use Exemption (CUE) process, a detailed procedure by which parties must demonstrate that alternatives are not available for a specific use, under the particular circumstances of the nomination. Since 2005, there has been a progressive trend by all parties to reduce their nominations for consumption for preplant soil uses and postharvest uses of methyl bromide, although this has occurred at different rates (TEAP, 2009). Figures 2 and 3 show the trends in the reduction in amounts approved/nominated for 'Critical Use' from 2005 to 2010 for strawberry and tomato crops in the major MB user regions in developed countries.

In A5 countries, phase-out has been achieved to a large extent through investment projects funded by the Multilateral Fund of the Montreal Protocol through its implementing agencies (UNIDO, UNEP and the World Bank) and some bilateral agreements (MBTOC, 2007; TEAP, 2009).

Substantial progress has been achieved in A5 countries that consumed the greatest quantities of MB. Since 2003, total A5 consumption has fallen by 1,420 metric tons per year on average (2003-2007). Only 12 parties still report consumption between 100 and 500 tons and only two countries remain in the usage category above 500 tons. Phase-out trends, per region, are illustrated in Figure 4.

CRITICAL USE EXEMPTIONS

Decision IX/6

Under Article 2H of the Montreal protocol, the production and consumption of MB is to be phased out in non-Article 5 countries by 1 January 2005. However, decision IX/6 established criteria allowing CUEs (TEAP, 2009). Production and consumption, if any, of methyl bromide for critical uses should qualify as "critical" only if the nominating party demonstrates that:

- The specific use is critical because the lack of availability of methyl bromide for that use would result in a significant market disruption;
- There are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environment and health and are suitable to the crops and circumstances of the nomination;
- Methyl bromide is not available in sufficient quantity and quality from existing stocks;
- All technically and economically feasible steps have been taken to minimise the critical use and any associated emission of methyl bromide;
- An appropriate effort is being made to evaluate, commercialise and secure national regulatory approval of alternatives and substitutes, taking into consideration the circumstances of the particular nomination and the special needs of the parties, including lack of financial and expert resources, institutional capacity, and information;
- That research programmes are in place to develop and deploy alternatives and substitutes.

The CUE Process

The exemption application process is extremely rigorous. Detailed information is required from each applicant, including comprehensive information on the impact of alternatives on crop yields and profit margins, and a description of efforts undertaken to develop, register, and apply new alternatives. The parties submit the CUN to the Ozone Secretariat of the United Nations Environmental Programme (UNEP). The Ozone Secretariat then forwards all CUNs to the Methyl Bromide Technical Options Committee (MBTOC). After analysis of the CUNs, MBTOC makes recommendations on the applications to the parties to the Protocol. The parties then determine whether or not to approve each application (TEAP, 2009).

In evaluating the CUNs for soil and postharvest treatments, MBTOC assumes that a technically feasible alternative to MB would need to provide sufficient pest control. Technically feasible alternatives do not necessarily provide superior pest control results than are achieved in practice by MB. When the requirements of Decision IX/6 are substantially met, MBTOC recommends the full amount of the request. Where some parts of a CUN do not meet Decision IX/6, MBTOC recommends a decreased amount, depending on its technical and economic evaluation. MBTOC reduces a nomination when a technical alternative is considered effective or, in a few cases, when the party failed to show that it was not effective. In cases where Decision IX/6 did not satisfy to a substantial extent, MBTOC does not recommend the nomination (TEAP, 2009)

Critical Use Nominations in the Dates Sector: Israel and USA

Most of the dates producing A5 and non-A5 countries have been using MB for about fifty years due its effect, efficient penetration, quick actions, low cost, highly killing capacity against the main date pests (Besri, 2008; Blumberg, 2008). The two countries presenting CUNs every year for dates'disinfestations are the US (California) and Israel

(TEAP, 2009).

1. Israel. Israel submitted CUNs in 2007, 2008 and 2009 for 2008, 2009 and 2010 uses. No nomination was submitted in 2010 for 2011 use. In the 2009 nomination, the most significant change since submission of previous nominations was the adoption of the thermal disinfestation treatment for the 'Mejhool' variety during the harvest season of 2008. This treatment was adopted in all the date packing stations. Investigations were then conducted to adopt the thermal disinfestation treatment to other varieties of dates. The results obtained from laboratory and field trials confirmed that this technology can also be used to disinfect all the dates'varieties. Heat technology is now providing a substitute for methyl bromide fumigation for 'Mejhool', 'Deglet Noor' and also for all the other varieties ('Hadrawi', 'Halawi', 'Deri' and 'Zehidi'). This is why no critical nomination was submitted in 2010 for 2011 use (Table 1).

From 2006 to 2009, the nominated MB quantities have been reduced from 3.444 to 1.560 tons (Table 1). Thermal disinfestation treatment for 'Mejhool' has been adopted during the 2008 harvest season at all date packing stations. In 2008 and 2009, this alternative was not adopted for the other varieties ('Amery', 'Deglet Noor', 'Hadrawi', 'Halawi', 'Deri' and 'Zehidi') because of difference in the handling procedure between 'Mejhool' and these varieties at the point of entry into the packing stations. 'Mejhool' dates are placed one layer high in shallow trays, to avoid the damaging of the soft 'Mejhool' dates. In contrast to 'Mejhool', the other varieties including 'Deglet Noor', are harvested and handled within the packing stations in plastic crates of 0.5-1 m^3 capacity for bulk storage of 200-400 kg of dates in which the dates are piled to a height of 40 cm. These crates have no sufficient ventilation to permit drying inside the crates. The thermo-physical aspects of the drying procedures inside crates and inside trays are completely different (Navarro, 2006). For the trays, the time needed for the dates to reach 50ºC is about 3 hours whereas for dates inside crates the required time is much longer. The conversion of handling the date varieties other than 'Mejhool', from crates into single layer trays appears as a costly operation not only in the replacement of the crates, but also in the entire handling system. In 2009 round, MBTOC recommended a reduced nomination of 1.040 tons, about 60% of the nominated amount (1.560 tons). The party nominated 1.560 tons and noted that methyl bromide is only used for those date varieties for which heat treatment or other alternatives have not been shown to be effective. The basis for the reduction in the nomination was to decrease the dosage rate to 20 g m^{-3} from 30 g m^{-3}.

2. USA. The US nomination for dates was for 'Deglet Noor' harvested in California. The moisture content of US dates at time of harvest is between 17-23%. The length of time needed to achieve date maturity on the tree, results in considerable drying, while the dates are still on the tree. Thus, US dates were referred to as 'fresh' but the American definition stands in contrast to the 'Deglet Noor' dates of North African countries (Algeria and Tunisia) which are also harvested 'fresh' at maturity but are at 30-40% moisture content. It is the moisture content and not the freshness of recent picking that impacts the potential for alternatives to be effective. When dates are at 17-23% moisture content, they are dry fruits (Navarro, 2006, 2009; Kader and Hussein, 2009), and for these fruits, alternatives exists (MBTOC, 2007). Heat, phosphine, controlled atmosphere and cold treatment are effective and are registered for use in the US. In addition, sulfuryl fluoride is also registered for treatment of dates and recent trials have indicated efficacy, at least for adults and larvae of some pests (Navarro, 2009). For these reasons, the US nomination was not recommended.

THE UNIDO PROJECT FOR ALGERIA AND TUNISIA

In Algeria and Tunisia, the main problem of the 'Deglet Noor' variety is its high moisture content which is between 30 and 40% (w/w). The main pest which infests dates in these two countries before and after the harvest is a *Lepidoptera*, the carob moth (*Apomyelois ceratoniae* = *Ectomyelois ceratoniae*).

In Decision XV/12, the parties to the Montreal Protocol recognise the risk of

potential non-compliance for those A5 countries that rely on the use of methyl bromide to stabilize and disinfest high moisture dates at time of harvest. Indeed, up to now, MBTOC has not been able to identify feasible alternatives to replace the use of this fumigant in the specific sector of high-moisture dates (MBTOC, 2007; TEAP, 2009). In the same decision the parties requested the Executive Committee of the Multilateral Fund to consider financing demonstration projects on alternatives for high-moisture dates. On behalf of the Governments of Algeria and Tunisia, UNIDO designed a project proposal to address the issue of alternative of MB for the palm date sector.

The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available in Algeria and Tunisia (UNIDO, 2008; Savigliano, 2009).

The project proposal was submitted to the 54th Meeting of the Executive Committee of the Multilateral Fund for the implementation of the Montreal Protocol for its consideration. The project was approved in April 2008 at the 54th Meeting of the Executive Committee, for a total cost of US$ 306,812 for the two countries, plus US$ 23,011 of support costs for UNIDO. The duration of the project is 24 months thus allowing tests in two consecutive harvesting seasons, namely in November 2008 and November 2009. Based on the actual needs of palm date producers and exporters and taking into consideration the locally available infrastructures, equipment and skills, the following five alternatives have been considered as potential alternatives to methyl bromide: heat treatment, ethyl formate, phosphine, modified atmospheres and sulphuryl fluoride (Ducom and Ciesla, 2009).

A workshop was organized in Vienna where scientific and technical experts have discussed the results of the experiments conducted in Biskra, Algeria (UNIDO, 2009): Controlled atmosphere is not compatible with the disinfestation of 'Deglet Noor' dates because the exposure time is so long that the dates ferment and become inedible. Phosphine with ammonia fumigation makes the dates darker and reduce the quality of the fruit. All other alternatives have potential to replace methyl bromide. Nevertheless, sulfuryl fluoride and ethyl formate cannot be considered for the next tests in full scale because they are not registered in Algeria. Therefore, the Vienna workshop recommended only two alternatives to be commercially tested: phosphine without ammonia (with an exposure time of 3 days and high temperatures) and heat treatment.

UNEP ACTIVITIES IN THE MIDDLE EAST

UNEP has organised many activities in the Middle East to discuss alternatives to MB for dates disinfestation (UNEP/ROWA, 2008; UNEP/ROWA, 2009). The Regional Workshop on "Uses of MB Alternatives in the Date Sector" held in May, 2008 in Cairo, Egypt, (UNEP/ROWA, 2008) recommended the member states to establish a database on date production in the region. The main objective of such a database is to assist the countries (1) accessing to a wide range of data about MB consumption in all sectors in general and in date sector and related industries in particular, (2) obtaining significant and reliable information about the trends in MB consumption in the date sector of each country, (3) identifying alternatives availability in each country which allow exchanging and transferring experiences as well as information sharing and (4) addressing their future needs of policies, regulations and legislations updating as well as research and/or assistance required (UNEP/ROWA, 2008). A regional experts group meeting on applications of methyl bromide alternatives in the dates sector was organised in 2009, in Al Khobar, Saudi Arabia (UNEP/ROWA, 2009). Before the meeting, a survey form was sent to technical experts experienced in MB and its alternatives use in the date sector in some regional dates producing countries (Iraq, Jordan, Saudi Arabia, UAE, Yemen, Egypt, Tunisia and Algeria). The surveys have shown that the date's situation in the Middle East is completely different from the one in North Africa. In Tunisia and Algeria, 'Deglet Noor' (which is not grown in the Middle East, except in Israel) has high moisture content, varying between 30 and 40%. In Jordan, only 'Mejhool' (semi dry) is fumigated. Egypt fumigates only dry varieties. In the surveys, phosphine as alternative has been

reported by Jordan, Egypt and Tunisia. Therefore, in the Middle East the date's fumigation problem is completely different from the one reported in Tunisia and Algeria. The approach to propose commercially and economically feasible alternatives should also be different (UNEP/ROWA, 2009).

The participants to the Al Khobar workshop recommended also to establish a "Regional Date/MB Helping Desk" to achieve the following objectives: 1) Establishment of a database to exchange knowledge among Arab countries in relation to date production and protection, 2) Organizing workshops and seminars for sharing information in relation to dates production and treatments, 3) Organise training (capacity building) in various fields of dates production and protection (preharvest and postharvest), 4) Editing a newsletter/journal on dates, 5) Helping scientists to address needed research areas, 6) Facilitating the creation of networks in the region to increase the scientific cooperation between the members countries, 7) Providing consultancies to the member states and to the private sector, 8) Raising awareness concerning the depletion of the ozone layer by MB and alternatives to this fumigant, 9) Develop a training handbook in the dates' sector. The MB Helping Desk will promote the date sector in all Arab countries and will provide solutions to the problems related to the use of MB alternatives faced by the date's producers.

CONCLUSIONS

The increasing demand for high quality dates that are not chemically treated and the phase-out of MB, which is used in fumigation and disinfestation of dates, have created the interest for searching and adopting alternatives. Two parties, Israel and USA, are regularly submitting CUNs in the date sector. Researchers in Israel have tested and developed an effective and practical alternative (heat treatment) to control pests in their date varieties under their packing house circumstances. Israel has not submitted any CUN in 2010 for 2011 use. MB has been completely phased out in the date sector in this country. It is unknown whether heat treatment would be suitable for fresh moisture dates in other countries. A UNIDO project will test this alternative in Algeria and Tunisia. The US 2009 CUN is for the 'Deglet Noor' variety (low moisture content). In its evaluation, MBTOC considered that alternatives exist and did not recommend this nomination. On behalf of the Governments of Algeria and Tunisia, UNIDO designed a project proposal to address the issue of alternative of MB for the palm date sector. The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available. The first results obtained are encouraging. UNEP/ROWA has organised many activities in the region to discuss and to implement strategies for phasing out MB in the dates sector.

Decision XVII/12 requests MBTOC to continually review progress in availability of alternatives for dates. Non-Article 5 countries should develop alternatives as soon as possible and before 2015. Cooperation between Non-Article 5 and Article 5 countries is needed. Funds from the MLF should be made available for A5 countries to develop alternatives.

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Tables

Table 1. Years of nomination, years of use, quantities nominated and recommended by MBTOC (TEAP, 2009).

Year of nomination	Year of use	Quantities (tons)	
		Nominated	Recommended
2005	2006	3.444	3.444
2006	2007	3.444	2.755
2007	2008	2.200	2.200
2008	2009	1.800	1.800
2009	2010	1.560	1.040
2010		No nomination	

Table 2. Methyl bromide historical use in the US dates sector (tons) (TEAP, 2009).

2003	2004	2005	2006	2007	2008	2009
2.616	2.468	2.887	3.145	1.999	2.019	0

From 2003 to 2008, The MB quantities used for the US dates'disinfestation varied from 3.145 tons (2003) to 2.019 tons (2008). The CUNs for 2009 use have not been recommended by MBTOC.

Figures

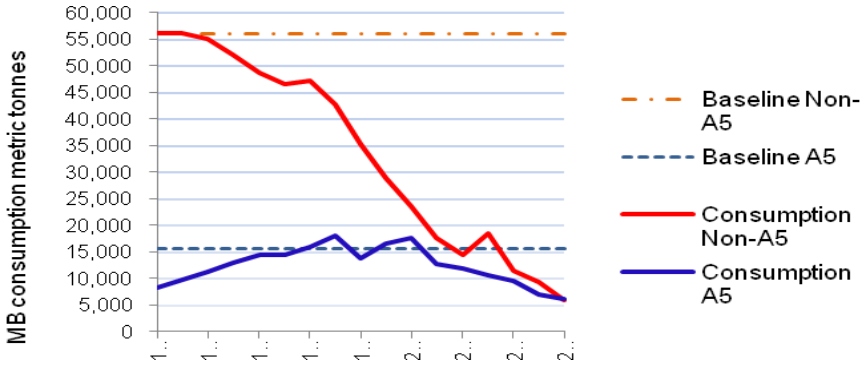
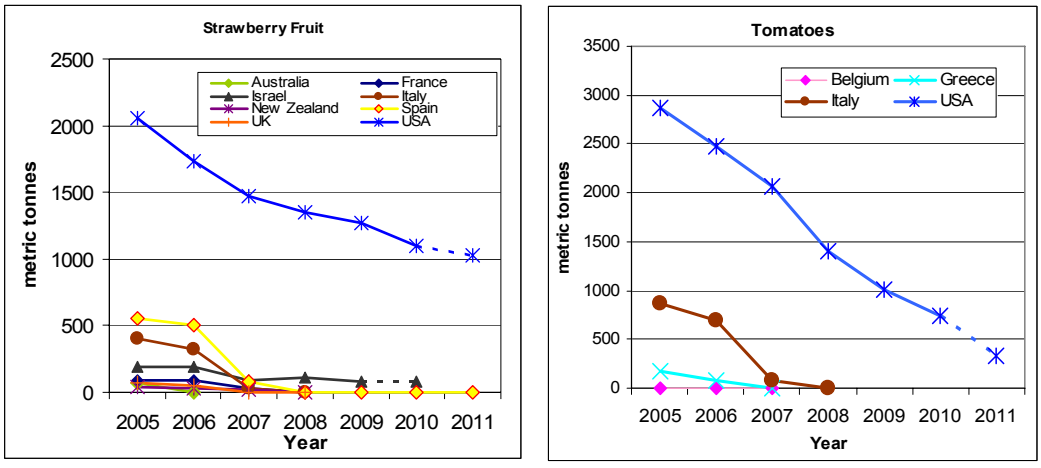


Fig. 1. Baselines and trends in MB consumption in Non-A5 (developed countries) and A5 (developing countries) 1991-2007 (metric tons). Source: MBTOC estimates calculated from Ozone Secretariat data at September 2009 (TEAP, 2009).



Figs. 2 and 3. Amounts of methyl bromide exempted for CUE uses in strawberry and tomato from 2005 to 2010 (TEAP 2009). Solid lines indicate trends in MB used for CUEs. Dashed lines indicate the quantity of methyl bromide nominated by the party in either 2010 or 2011 (TEAP, 2009). CUEs: Critical Use Exemptions.

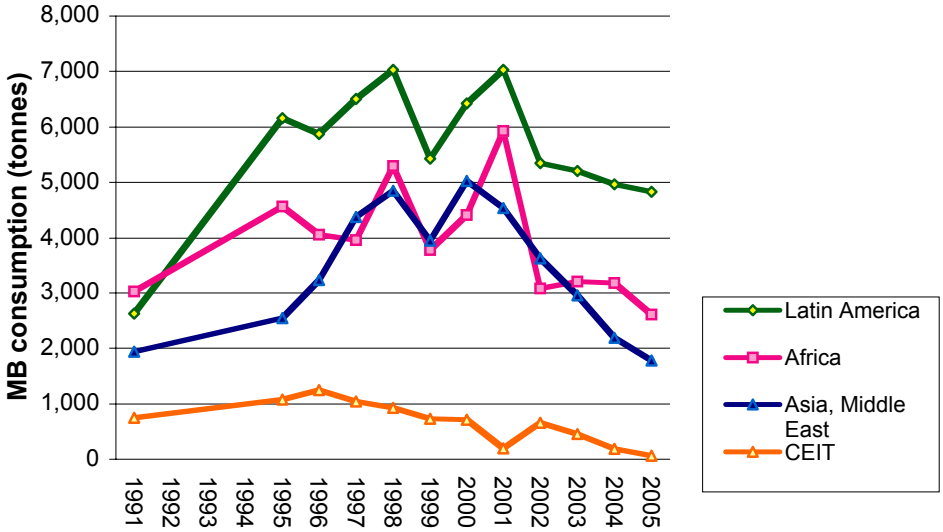


Fig. 4. Methyl Bromide trends in A 5 and CEIT countries 1991 – 2007 (TEAP 2009). Source: Ozone Secretariat data, 2009. CEIT : Countries with Economies in Transition (TEAP, 2009)

Effect of Moisture Content on Dates Disinfestation with Methyl Bromide

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Keywords: dates' fruits, date's development, moisture content, methyl bromide, Montreal Protocol.

Abstract

Dates are infested by insects before, during and after harvest. Dates fumigation with methyl bromide upon arrival at the packing houses, controls infestation effectively and also causes a high proportion of larvae and adults to emigrate from the fruit before they die. Because of its contribution to stratospheric ozone layer depletion, this fumigant has been phased out in developed countries in 2005 (Non-Article 5 countries) and it will be phased out in developing countries (A5 countries) by 2015. However, its use in non-Article 5 countries is still permitted under the Critical Use Exemption (CUE) control. Dates, during their development, go from maximum moisture content (85%) at the early kimri and early khalaal stages to less than 30% at tamr stage. Up to now, the Methyl Bromide Technical Options Committee (MBTOC) has not been able to identify feasible alternatives to replace the use of this fumigant in high-moisture dates' disinfestation. The parties to the Montreal Protocol requested the Excom of the Multilateral Fund to consider financing demonstration projects on alternatives for high-moisture dates. MB alternatives to semi-dry or dry varieties should also be developed because their harvest is delayed until ripening on the tree that makes them suitable for nitidulid beetles attacks. A date's variety could be considered as semi dry or as soft (high moisture date) depending on its origin and growing conditions.

INTRODUCTION

Methyl bromide (MB) is used as an effective broad spectrum fumigant for pest control in agriculture, disinfestation of both durable and perishable commodities and for quarantine and pre-shipment (QPS) uses (TEAP, 2009). Having recognized the environmental challenges and threats of this fumigant (Molina and Rowland, 1974), the international community reached a consensus in 1997 on MB global phase-out as an ozone depleting substance under the Montreal Protocol (MP). The MP's schedule requires that developed countries phase-out MB by 2005 and developing countries by 2015 (MBTOC, 2007; TEAP, 2009).

Although many sectors in industrialized countries met the phase-out of 2005, a large number, including high moisture dates, are still using MB under the Critical Use Exemption (CUE). MBTOC has not identified available and technically effective alternatives for high-moisture fresh dates (MBTOC, 2007; TEAP, 2009). Accordingly, date producers, almost in all producing countries, use MB for prevention and containment of insect infestation as its use is still permitted in this important sector under the CUE control in developed countries (MBTOC, 2007; TEAP, 2009; Besri, 2008a, 2009a). According to a survey conducted in the some Arab dates producing countries (Besri, 2009b) MB is also used on semi dry and dry dates. Therefore, the problem of technically and feasible alternatives in the dates sector is a problem of all dates and not only for high moisture dates (Besri, 2009b). MB alternatives to semi-dry or dry varieties should also be developed because their harvest is delayed until ripening on the tree which makes them suitable for nitidulid beetles attacks.

Following the difficulty in finding viable MB alternatives for high-moisture dates,

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the parties to the Montreal Protocol requested the Excom of the MLF to consider financing demonstration projects on alternatives for high-moisture dates (UNIDO, 2008).

The aim of this paper is to present the moisture content evolution according to the dates' development, dates' harvesting and air humidity. The paper will also focus on major dates' pests infesting the fruit before and after harvest, on high moisture dates' disinfestation and stabilisation with MB, on the effect of dates' moisture on insect infestation, and finally, on MB uses in some developing and developed countries.

DATE FRUIT DEVELOPMENT

Dates take up to about 200 days from pollination to reach full maturation (tamr). During its formation and ripening, the fruit passes through 5 stages. Stage I (hababouk), stage II (kimri), stage III (khalaal), stage IV (rutab) and stage V (tamr) (Table 1) (Dowson, 1962; Barreveld, 1993; Kader and Hussein, 2009).

Table 1 shows that dates go from maximum moisture content (85%) at the early kimri and early khalaal stages to less than 30% tamr stage. Between these maximum and minimum, there are other moisture levels: 45 % for late khalaal stage, 30-45% for rutab and less than 30% for tamr. 'Deglet Nour' and 'Mejhoul' are considered as high moisture content varieties when harvested at the rutab stage and as semi dry dates when harvested at the tamr stage.

Names of the fruit stages vary from one country to another (Table 2). In this paper, we will use the most common names adopted in the Middle East. However, it is recommended to use the date's stages instead of the local names (UNIDO, 2009; UNEP/ROWA, 2009).

To better understand the effect of moisture dates on methyl bromide fumigation, it is important to describe the different stages of the date's development and the evolution of the moisture content before and during harvest.

Stage I: Hababouk

Hababouk is the term used for the female flower and the period just after pollination when the young fruit is still creamy white before gradually turning green at the kimri stage. This stage starts soon after fertilisation and continues until the beginning of stage II (kimri). It usually takes four to five weeks to complete. Fruit at this stage is immature and is completely covered by the calyx and only the sharp end of the ovary is visible. Its average weight is one gram and the size is about that of a pea. The moisture content is higher than 85% (Dowson, 1962; Barreveld, 1993; Glasner et al., 2002; Navarro, 2009; Besri, 2009d; Kader and Hussein, 2009).

Stage II: Kimri

At this stage there is a rapid increase in size, weight and reducing sugars. It is the period of highest acid activity and moisture content (up to 85%). At this point the date seed could already germinate and the fruit is botanically mature. This stage lasts from a small green berry to an almost full sized green date. It is the longest stage of growth and development of dates (Barreveld, 1993; Glasner et al., 2002; Navarro, 2006; Besri, 2009d; Kader and Hussein, 2009).

Stage III: Khalaal

The fruit is physiologically mature, hard ripe and the colour changes completely from green to greenish yellow, yellow, pink, red or scarlet depending on the variety. It lasts three to five weeks depending on varieties. At the end of this stage, date fruit reaches its maximum weight and size, but sugar concentration (saccharose), total sugar and active acidity have a rapid increase associated with a decrease in water content (around 45-85%). At this stage colour of the seed changes at the end from white to brown. A few date cultivars such as 'Barhee', 'Hallawi', 'Hayani', 'Samany' and 'Zaghloul' are harvested at this stage (partially ripe) when they are yellow or red (depending on cultivar), but many consumers find them astringent (due to the high tannin content). Ripening of khalaal dates

can be hastened by bunch bagging during growth. After harvest, these dates can be ripened to the rutab stage by either quick freezing and keeping at -18°C or lower temperatures for at least 24 hours and thawing them, or by exposure to acetaldehyde or ethanol vapour. Dates at the khalaal stage must be eaten immediately after harvesting as they will keep for only a few days without cold storage (7°C for one week or 0-1°C for longer periods) due to their high sugar and water content which cause fermentation during hot weather (Barreveld, 1993; Glasner et al., 2002; Navarro, 2006; Besri, 2009d; Kader and Hussein, 2009). Fresh cultivars are not fumigated and therefore it is not necessary to develop alternatives to MB for these cultivars (Navarro, 2010).

Stage IV: Rutab

Some dates are harvested at the fully-ripe rutab stage(light brown and soft). At this stage, the tip at the apex starts ripening, changes in colour to brown or black and becomes soft. It begins to lose its astringency and starts acquiring a darker and less attractive colour from the previous stage. At this stage, which in total lasts for 2 to 4 weeks, there is a continuous decrease in fresh fruit weight mainly due to loss of moisture (Barreveld, 1993; Glasner et al., 2002; Navarro, 2006; Besri, 2009d; Kader and Hussein, 2009). The moisture content at this stage varies from 30 to 45%. Most of the fumigated date varieties, particularly 'Deglet nour' (Tunisia, Algeria, and Israel) and 'Majhoul' (Israel) are fumigated at this stage (Besri, 2009b,c).

Stage V: Tamr

This is the stage when the dates are fully ripe. The colour of the skin and of the underlying flesh darkens with time. At this stage, the date contains the maximum total solids and has lost most of its water to such an extent (below 30% down to 10% and less) that it makes the sugar/water proportion sufficiently high to prevent fermentation (Barreveld, 1993; Glasner et al., 2002; Navarro, 2006; Besri, 2009c; Kader and Hussein, 2009). This is the best condition for storage without any fumigation. The loss in fruit weight continues if fruits, such as 'Deglet Nour' in the US (California), are left on the palm. This stage is equivalent to that of the raisin in the grape and the dried prune in the prune type of plum. In Jordan, 'Majhoul' is fumigated with MB at this stage (UNEP/ROWA, 2009).

Harvesting, Moisture Content and Perishability

As previously reported in Table 1, dates could be harvested and marketed at three stages of their development (Khalaal, Rutab, and Tamr). The choice for harvesting at one or other stage depends on varietal characteristics, climatological conditions and market demand. The most common harvesting stages are rutab and tamr (Barreveld, 1993; Zaid, 2002; Navarro, 2006).

Humidity of the air, expressed as a relative percentage is of great importance to the moisture content of the final products because it is a measure of the absorptive moisture capacity of the air (Navarro, 2006, 2009). The relation between the air relative humidity and moisture content is rather simple. In regions with low relative humidity, dates tend to dry out on the palm until they are hard with moisture content as low as 10% (Fig. 1). On the other hand, high relative humidity delays the evaporation of moisture to the extent that the dates do not reach the adequate moisture content for preservation and have to be harvested at a perishable stage (Navarro, 2006).

The Equilibrium Moisture Content (EMC) expresses the water activity and the equilibrium between moisture date content and relative humidity (Navarro, 2006). Therefore, the EMC helps in evaluating the sensitivity of the fruit to microbiological infestation. EMC below 65% ensures resistance to microbiological factors such as moulds, yeast and bacteria that attack the fruits, but does not prevent from insect infestation. A moisture content of 24% (tamr stage) is in equilibrium with surrounding air of 70% relative humidity. Considering that moulds are unable to grow in an atmosphere below 70% relative humidity, for yeasts and bacteria even higher, the tamr stage should

be considered not perishable. Instead, rutab at 35% is above this level and must be considered perishable. The curve is further useful when considering storage conditions and drying of dates. Moisture content can be artificially manipulated by drying (either in the sun or by dehydration) to remove, or by (vacuum) hydration and steaming, to add water (Navarro, 2006).

METHYL BROMIDE DATES DISINFESTATION AND STABILIZATION

Major Pests of Date Palms Fruits

Insect infestation and damage caused by insect feeding on the dates is one of the primary causes of postharvest losses in quality and quantity. The typical climate during the date harvesting season (i.e., hot and quite often humid) are ideal for insect infestation to occur (Blumberg, 2008; Kader and Hussein, 2009). The postharvest insects infesting dates are also field pests. Therefore, the initial source of infestation is frequently on ripening fruit on the tree and infestation continues during process and storage (Table 3).

Effects of Date's Moisture on Insects Infestation

Insects' development in storage is favoured by three main factors: a) high initial degree of infestation prior to storage, (eggs), b) elevated temperatures and humidity of the air, c) higher moisture level of the date. Insect pests depend on their food supply to obtain the moisture they require for their life processes. Up to a certain point, the higher the moisture of the dates, the higher the rate of increase of insect pests. Above the "critical moisture content" where moulds are able to develop, there is a negative effect on the quality of the food supply that in turn affects insect development (Barreveld, 1993; Glasner, 2002; Navarro, 2006).

Some insects are mainly associated with dates of high moisture content, whereas others develop also in fruit of low moisture content (Navarro 2006; Kader and Hussein, 2009). Thus, the harvested crop is rapidly treated upon arrival at the packing shed in order to control insect pests. This is presently an accepted practice in the date industry as it is in the case of other dried fruits and nuts, such as figs. Dates that will be stored for long periods (several months to one year) must be completely clean of any pest stages that could be present (eggs, pupas, larva or adults) (Navarro, 2006; Blumberg, 2008; Kader and Hussein, 2009).

Methyl Bromide Use in Some Regions and Countries

Some date-producing countries (e.g., Algeria and Tunisia, USA, Israel, Jordan, Egypt) currently use MB for rapid disinfestation and stabilization of high moisture and semi dry dates (rutab and tamr stages, humidity > or around 30%) at time of harvest. MB is used by the date industry for technical and economical reasons: treatment is easy to perform, is not expensive, is very quick and additionally causes a high proportion of larvae and adults to emigrate from the fruit before they die (emigration effect). This emigration phenomenon is not less important than the toxic effect of the treatment because minimum acceptance tolerances have been set for the presence of both dead and live insects in dried fruits (Barreveld, 1993; Glasner et al., 2002; Navarro, 2006). Fumigation with MB is mostly done for the high-value varieties (principally 'Deglet Nour' or 'Medjool'), which are intended for export. Fumigation is required to prevent deterioration after harvest (stabilization), to kill any pests present at harvest that may subsequently cause loss of quality and to disinfest in order to meet the sanitary standards set by importing countries. In the past, treatment was conducted with ethylene oxide but this is no longer permitted because of residue and health considerations (Besri, 2008c; Navarro, 2006; Kader and Hussein, 2009).

1. North Africa: The UNIDO Project. In North Africa (Algeria and Tunisia), the main problem of the 'Deglet Nour' variety is its high moisture content which is between 30 and 40% (w/w) (UNEP/ROWA, 2009; UNIDO, 2009). As a result, the exposure time of fumigation must be very quick to reduce the risk of fermentation during the treatment.

The main pest which infests dates in Algeria and Tunisia before and after the harvest is a *Lepidoptera*: the carob moth (*Apomyelois ceratonia = Ectomyelois ceratoniae*). Algeria and Tunisia have discussed with deep concern the problem of controlling pests in high moisture dates. In 2007, MBTOC noted that technically and economically effective alternatives have not been identified for fresh, high-moisture dates. The parties then passed Decision XV/12 which noted the problem and its resulting impact on MB use in those countries. The Decision also indicated a need for a project to identify suitable alternatives and a workshop to share this information.

On behalf of the Governments of Algeria and Tunisia, UNIDO designed a project proposal to address the issue of alternative of MB for the palm date sector. The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available in Algeria and Tunisia (Ducom and Ciesla, 2009; Savigliano, 2009). The project proposal was submitted to the 54th Meeting of the Executive Committee of the Multilateral Fund for the implementation of the Montreal Protocol for its consideration. Based on the actual needs of palm date producers and exporters and taking into consideration the locally available infrastructures, equipment and skills, the following five alternatives have been considered as potential alternatives to methyl bromide in Algeria and Tunisia: heat treatment, ethyl formate, phosphine, modified atmospheres and sulphuryl fluoride (Ducom and Ciesla, 2009; Savigliano, 2009).

After one year of experiments, UNIDO held a workshop where scientific and technical experts discussed the results of the experiments conducted in Biskra, Algeria (UNIDO, 2009). The main results obtained during the first year of experiments are:

- Controlled atmosphere is not compatible with the disinfestation of these dates because the exposure time is so long that the dates ferment and become inedible.
- Phosphine with ammonia fumigations make the dates darker and reduce the quality of the fruit.
- All other alternatives have potential to replace methyl bromide. Nevertheless, sulfuryl fluoride and ethyl formate cannot be considered for the next tests in full scale because they are not registered in Algeria.

Therefore, the workshop recommended only two alternatives to be commercially tested: phosphine without ammonia (with an exposure time of 3 days and high temperatures) and heat treatment (Ducom and Ciesla, 2009; Savigliano, 2009) .

Morocco is not involved in this project. MB is not used for date's disinfestation in this country (Besri, 2008e).

2. USA. The US dates moisture content ('Deglet Nour') at time of harvest is between 18-23%. The length of time needed to achieve date maturity on the tree results in considerable drying, while the dates are still on the tree (TEAP, 2009). Thus, US dates were referred to as 'fresh', but the American definition stands in contrast to the 'Deglet Nour' dates of North African countries which are also harvested 'fresh' at maturity but are at 35-40% moisture content. Dates at 18-23% moisture content are considered as semi dry fruits. In the US, the word 'fresh' is a marketing term. Heat, phosphine, controlled atmosphere and cold treatment seem likely to be effective and are registered for use in the US (TEAP, 2009).

3. Israel. In Israel some cultivars e.g., 'Barhee' (yellow) and 'Hayany' (red) are harvested at the khalal stage when the fruit is at high moisture content and partially-ripe. They are called 'fresh' varieties since their maturation takes place at low temperatures and they are consumed while their moisture content is very high 46-65%. All other date cultivars ('Medjool', 'Deglet Noor', 'Amry', 'Dayri', 'Zahidi', 'Halawy' and 'Khadrawy') are semi dry or dry while their ripening takes place on the tree during the drying stage. As a result their attack by nitidulid beetles is most common (Navarro, 2010).

Thermal disinfestation of high moisture dates is now adopted in all the date packing stations in Israel. During the last years, investigations were conducted to adopt the thermal disinfestation treatment to all the date varieties e.g., 'Deglet Nour', 'Majhoul', 'Hadrawi', 'Halawi', 'Deri' and 'Zehidi' (Navarro, 2006; TEAP, 2009).

4. Middle East. A regional experts group meeting on applications of methyl bromide alternatives in the dates sector was organised by UNEP/ROWA (2009). Before the meeting, a survey form was sent to technical experts experienced in MB and its alternatives use in date sector in some regional dates producing countries (Iraq, Jordan, Saudi Arabia, UAE, Yemen, Egypt, Tunisia and Algeria). The surveys have shown that the date's situation in the Middle East is completely different from the one in North Africa. In Tunisia and Algeria, 'Deglet Nour' (which is not grown in the Middle East, except in Israel) has high moisture content, varying between 30 and 40%. In Jordan, only 'Mejhool' (semi dry) is fumigated. Egypt fumigates only dry varieties. Therefore, in the Middle East the date's fumigation problem is completely different from the one reported in Tunisia and Algeria. The approach to propose commercially and economically feasible alternatives will also be different (UNEP/ROWA, 2009). MB alternatives should also be developed for semi dry or dry varieties because their harvest is delayed until ripening on the tree which makes them suitable for nitidulid beetles attacks.

CONCLUSIONS

Dates take up to about 200 days from pollination to reach full maturation (tamr). During its formation and ripening, the fruit passes through 5 stages: hababouk, kimri, khalaal, rutab and tamr. The date's moisture content goes from 85% at the early kimri and early khalaal stages to less than 30% at tamr stage. Between this maximum and minimum, there are other moisture levels: 45% for late khalaal stage, 30-45% for rutab and less than 30% for tamr. Dates could be harvested and marketed at three stages of their development (khalaal, rutab and tamr). The choice for harvesting at one or other stage depends on varietal characteristics, climatological conditions and market demand. Fresh cultivars at khalaal stage are not fumigated and therefore it is not necessary to develop alternatives to MB for these cultivars. The most common harvesting stages are rutab and tamr. Dates at rutab stage (30-45%) are considered perishable. Dates at tamr stage (less than 30 %) are not perishable. The typical climate during the date harvesting season (i.e., hot and quite often humid) are ideal for insect infestation to occur. The postharvest insects infesting dates are also field pests. Therefore, the initial source of infestation is frequently on ripening fruit on the tree and infestation continues during process and storage. Insects' development in storage is favoured by many factors particularly by moisture level of the date. Up to a certain point, the higher the moisture of the dates, the higher the rate of increase of insect pests.

Dates that will be stored for long periods (several months to one year) must be completely clean of any pest stages that could be present (eggs, pupas, larva or adults). A UNIDO project for Algeria and Tunisia considered five potential alternatives to MB: heat, ethyl formate, phosphine, modified atmospheres and sulphuryl fluoride. After the first year of experiment, only two alternatives will be commercially tested in the future: phosphine and heat treatment. The US dates moisture content ('Deglet Nour') at time of harvest is between 18-23%. For these dates, heat, phosphine, controlled atmosphere and cold can be used as alternatives to MB. In Israel, thermal disinfestation is now adopted in all the date packing stations. In Jordan, only 'Medjool' (semi dry) is fumigated. Egypt fumigates only dry varieties. Therefore, in the Middle East (except Israel),the date's fumigation problem is completely different from the one reported in Tunisia and Algeria. The approach to propose commercially and economically feasible alternatives should also be different. MB alternatives should also be developed for semi dry or dry varieties because their harvest is delayed until ripening on the tree which makes them suitable for nitidulid beetles attacks.

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Tables

Table 1. Development stages and moisture content of a date fruit during its maturation from stage I (Hababouk) to Stage V (Tamr).

Stage	Varieties harvested and consumed	Moisture content (%)
StageI: hababouk	None	>85
Stage II: kimri	None, fruits are immature.	85
Stage III: khalaal	Barhee, Hallawi, Hayani and Zaghloul, Samany. Must be eaten immediately after harvesting.	Early stage : 85 Late stage: 45
Stage IV: rutab	Many varieties. Fruit is very sweet. Unless they are cold stored, the fruits quickly turn sour and become of no commercial value: Deglet Nour, Mejhoul.	Tip browning : 45 50% browning :40 100% browning : 30
Stage V: tamr	Dates are fully ripe, the texture of the flesh is soft, best condition for storage: Deglet Nour, Mejhoul.	Less than 30

Adapted from Barreveld, 1993; Kader and Hussein, 2009.

Table 2. Names of the date's stages according to the country (Besri, 2009c).

Country	Stage I	Stage II	Stage III	Stage IV	Stage V
Morocco	Lilou	Bourchime	Bleh	Nakkar or Rteb	Tmar
Algeria	Loulou	Khelal	Bser	Martouba or Mretba	Tmar
Mauritania	Zei	Tefejena	Enguei	Bleh	Tmar
Libya	-	Gamag	Bser	Routab	Tmar
Middle east	Hababouk	Kimri	Khalal	Routab	Tmar
Stage duration (weeks)	4-5	7-14	3-5	2-4	2-3

Besri, 2009c.

Table 3. Major pests of date palm fruits (Blumberg, 2008).

Pest species	Common names	Green fruit	Ripe fruit
1. *Palmapspis* (*Asterolecanium*) *phoenicis*	Green scale	+	+
2. *Parlatoria blanchardi*	Parlatoria scale	+	+
3. *Dysmicoccus brevipes*	Pineaple mealybug		+
4. *Carpophilus* spp.	Sap beetles		+++
5. *Coccotrypes dactiliperda*	Date stone beetle	+++	
6. *Cadra* (*Ephestia*) spp.	Raisin , fig, moths		+++
7. *Spectrobates* (*Ectomyelis*) *ceratoniae*	Carob moth		+++
8. *Arenipses sabella*	Greater date moth		++
9. *Badrachedra amydraula*	Lesser date moth	+++	
10. *Raoiiella indica*, *Phyllotectranychus* sp. and others	Date mites	+++	

+: less preferred; +++: most preferred.
Hemiptera (1,2,3); *Coleoptera* (4,5); *Lepidoptera* (6,7,8,9); *Acarina* (10).

Figures

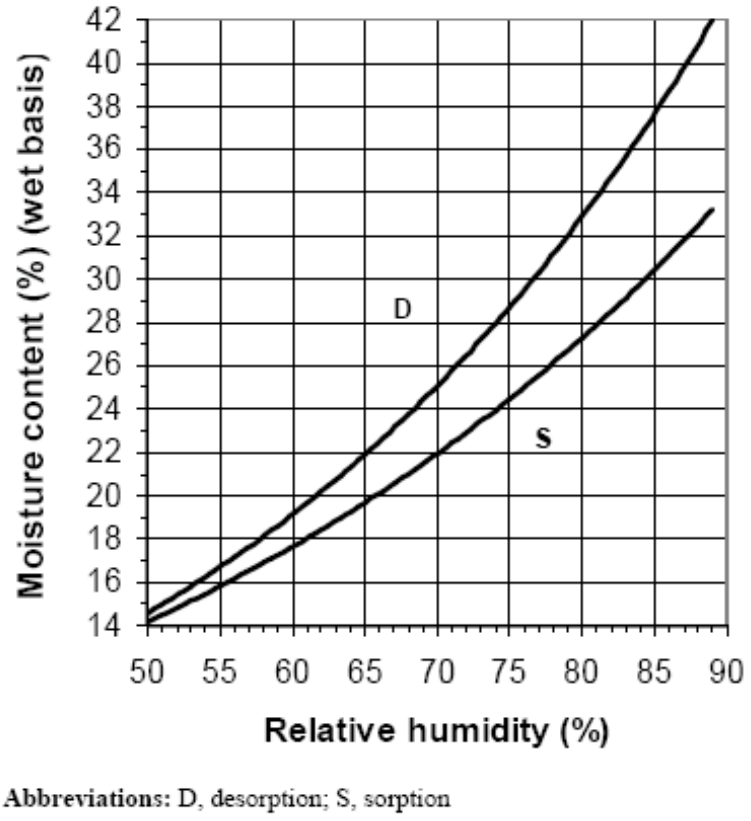

Fig. 1. Equilibrium between the air relative humidity and moisture content (%) (Wet-basis) of the 'Majhool' date variety at 26°C (Navarro, 2006).

Methyl Bromide Alternatives for Dates Disinfestations

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Keywords: modified atmosphere, phosphine, heat treatment, ethyl formate, irradiation, combined mixture, combined application

Abstract

Methyl bromide (MB) is a fumigant that has been used to control a wide range of pests in agriculture and for disinfestations of durables and perishable commodities. At the moment, MB is the main fumigant in use for dates' disinfection. Fumigation must not be carried out when the fruit is fresh, harvested at the khalal stage or when stored under deep refrigeration. The average practical dose is 15 g/m^3 for 12-24 hours at 15-16°C. However, MB is one of the most powerful chemicals that deplete the stratospheric ozone layer. In 1997, the Meetings of the Parties required that developed countries phase out MB by 2005 and by 2015 in developing countries. According to 2006 Assessment Reports of the UNEP's MB Technical Options Committee, alternatives to MB have been identified for about 95% of controlled uses. However, no technically and economically effective alternatives are identified for high-moisture dates. Many feasible alternatives to MB for dates' disinfestations are known of which are heat treatments, heat and carbon dioxide, phosphine (PH_3), sulfuryl fluoride, ethyl formate, modified atmosphere and phosphine/CO_2 mixture. In this respect, two laboratory experiments were conducted to evaluate the effect of modified atmospheres (MAs) as well as MAs/PH_3 mixture on controlling *Oryzaephilus surinamensis* and *Tribolium confusum* in stored dates. The results of the first experiment indicated that application of MAs alone achieved 100% mortality of tested pests after 36-48h, depending on the type of the pest and CO_2 concentration. The second experiment indicated that 100% mortality of tested pests' stages was achieved after 6h only when CO_2 was combined with half the recommended dose of PH_3. It can be concluded that the use of CO_2 in combination with PH_3 significantly shortened the time required to achieve complete mortality of infesting pests. Simultaneously, this treatment did not cause any noticeable changes in the tested chemical properties of treated fruits.

INTRODUCTION

Date palm cultivation and production are concentrated mainly in South West Asia and North Africa where arid regions and high temperature climates prevail. In 2007, the estimated global date production was about 6,638,774 tons. Two thirds of the total world date production (63.3%) comes from Asia whereas one third (36.2%) comes from Africa (FAO, 2007). However, the date production is really in danger due to (1) spread of insects and pathogens that infect this important crop in every growth stage including postharvest pests and (2) the lack of projects and researches that aim to develop date palm cultivation and production, preserve the current cultivars and develop new cultivars through new agricultural technology.

Methyl bromide (MB) has globally been used as an effective broad spectrum fumigant for pest control in agriculture, disinfection of both durable and perishable commodities, controlling postharvest pests and for quarantine and pre-shipment (QPS) applications. According to the UNEP's Scientific Panel (http://www.unep.org/roa/Projects_Programmes/ozone/MB/About/Intro.asp), it is estimated that MB is responsible for approximately 5 to 10% of worldwide ozone depletion with ozone depleting potential

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of 0.6. Having recognized the environmental challenges and threats, the international community reached a consensus in 1997 on MB global phase-out as an ozone depleting substance under the Montreal Protocol. The Montreal Protocol's schedule requires that developed countries phase out MB by 2005 and developing countries by 2015.

One of the challenges of the last decade has been the ongoing need to develop and implement alternatives to MB for all its diverse uses. The fact that MB cannot generally be replaced by one in-kind alternative has become clear through both demonstration and investment projects, thus combination of practices or treatments will often be required (Batchelor, 2000). This has resulted in farmers and pest control operators having to learn new ways of controlling pests and, unlike most chemical control methods, these new ways are knowledge and capital intensive.

According to the 2006 Assessment Report of the UNEP's MB Technical Options Committee (MBTOC), alternatives to MB have been identified for about 95% of controlled uses. However, MBTOC has not identified available and technically effective alternatives for high-moisture fresh dates (UNEP, 2006). Accordingly, date producers, almost in all producing countries, use MB for prevention and containment of insect infestation as its use is still permitted in this important sector, even in non-Article 5 countries (Critical Uses Nominations). Following the difficulty in finding viable alternatives for stabilizing high-moisture dates, the 15th Meeting of the Parties in its 12th Decision recognized that Parties which consume over 80% of their MB for high-moisture dates cannot meet the Protocol's MB control schedule without production losses for that important cash crop for their countries. Hence, the compliance status of these countries is deferred until two years after the Technology and Economic Assessment Panel (TEAP) formally finds that there are alternatives to MB available for high-moisture dates.

The Regional Office for West Asia (ROWA) recognized this critical issue and consequently, through its Compliance Assistance Programme (CAP), organized three workshops (Cairo 2007, Cairo 2008 and Al-Khobar 2009) to discuss MB applications and its alternatives in this vital sector. The main objective of these workshops were to help the dates producing countries define their needs for gradual MB phasing out in the date sector before 2015, the date of the total phasing out of this substance in developing countries. The outcomes of those workshops indicated clearly that (1) world dates production increases dramatically as a result of growing new varieties of high productivity, application of developed agricultural practices and increasing interest of many countries to include date palm cultivation in their agricultural policies, (2) MB consumption has increased in the past few years (2005-2008) and is expected to exacerbate in the next 15 years as all dates producers will have to store the majority of dates production for almost a year, particularly in the Arab region, as Ramadan month is coming before harvesting time, (3) the high temperature in these countries along with the long period of storage make the stored amounts more vulnerable to infestation by various pests and insects causing the need for more fumigation treatments and (4) some countries consume large quantities of MB for disinfestations of semi dry and dry varieties as these varieties play important social and economic roles. Consequently, MB use is expected to increase dramatically in the future (UNEP/ROWA, 2010, in press).

The present paper aims to highlight the current status of MB alternatives available for dates disinfestations.

WHAT IS THE ALTERNATIVE?

MBTOC has defined the alternatives as "any practices or treatments that can be used in place of MB". The 'existing alternatives' are those alternatives in present or past use in some regions whereas the 'potential alternatives' are those in the process of investigation or development (UNEP, 2006). In dates industry, there are some factors that may cause a potential alternative to MB unsuitable for dates disinfestations such as (1) technical and economical infeasibility of the alternative, (2) geographical and climatic variations between different areas where date processing facilities are located, (3) limitations of some alternatives due to its effect on characteristics of the final products

and (4) negative economic impact to the producers and processors as alternative fumigants often require more time compared to MB.

Based on the available information, few ongoing researches and projects are taking place in some date producing countries to reach viable, efficient and cost effective alternatives for dates treatment, particularly for high moisture dates. However, no recommendations are available to apply certain alternative(s) on commercial scale, so far.

Methyl Bromide Alternatives for Dates Disinfestations

1. Cold Treatment. Storage at 0.0 and -5°C is relatively inefficient for control of *Carpophilus* spp., particularly since rates of cooling of the dates, and the form and size of packaging, must be taken into consideration (UNEP, 2002). Cooling to very low temperatures (-10 to -18°C) is an established system for disinfestations of dates, replacing MB treatment. It is most effective when combined with a short exposure to low pressure or 2.8% oxygen, which causes insects to leave the centre of the fruit (Donahaye et al., 1992), making them vulnerable to the cold treatment. It has been stated that an exposure time of 10.5 hour to -10°C or 2.25 hour exposure to -18°C killed all stages of the relevant insect pests (Donahaye et al., 1991). However, cold treatment is highly energy consuming.

2. Heat Treatment. Heat is used as disinfestations treatment of dry and durable food products against pests, insects and microorganisms. This technology does not pose significant risks to human health or his surrounding environment as heat can be generated by different means of which electrical, steam and propane heaters (AlterBromide, 2008).

Heat treatment technologies for disinfestations of fruits have been accepted by the US Department of Agriculture (USDA) as disinfestation treatment against pests for perishables (APHIS, 1993) and are used as disinfectant against Mediterranean fruit fly (Couey, 1989) and stored products (Roesli et al., 2003). Some hypotheses illustrated the mode of action of heat treatment on pests that include phospholipids membranes become more fluid, the structure of proteins and hence enzymes are adversely affected by high temperature, pH is temperature dependent, and water stress may be a crucial factor between 35 and 43°C (Paul and Noel, 2002). Recently, heat is used for the treatment of dates, and can be combined with CO_2, to control postharvest pests as it proved to be applied as an alternative to MB in this industry (AlterBromide, 2008). In industrial drying processes of dates, temperatures are usually kept moderate (35-55°C) to avoid discolouration and blistering effect, the phenomena that separates the skin from the flesh of the fruit. Meanwhile, in a laboratory study, Finkelman et al. (2006) carried out an experiment to control *Carpohyilus hemipterus* larvae at time of harvest. The results indicated that 2h of exposure to 50°C resulted in 100% mortality and 92.3% of the total pest number migrated from the fruits. Rafaeli et al. (2006) reported that exposing date fruits to 55°C for 2.5h was an optimum temperature regime for maximum escape of sap-beetle (*Coleoptera*: *Nitidulidae*) from the fruit. The emigration of pests from treated date fruits is of importance for achieving the minimum acceptable limits for insect infestation in the treated fruits.

However, according to Ducom and Ciesla (2008), dates ('Deglet Noor') treatment at 50°C for 2h caused 100% mortality of targeted pest but also caused a noticeable dry skin. These findings clearly indicate that the marketing properties of each variety should be considered before choosing heat treatment as an alternative application to MB.

3. Carbon Dioxide. Some studies were carried out to evaluate storing dried dates under CO_2 atmosphere as an alternative to MB. CO_2 (60-80%) was used to treat dates stored in the chamber in bulk or packed on pallets containing different dates varieties (Navarro et al., 2001). The results indicated no significant difference in terms of dates' quality between the treated dates and controls kept at -18°C. The insect population was effectively controlled. On the other hand, studies under laboratory conditions (Navarro, 1988) and in field tests at ambient temperatures (Navarro et al., 1995) showed that CO_2 significantly delayed browning and sugar formation on dates, and extended shelf life.

4. Heat and Carbon Dioxide. Heat alone, in the range of 45-50°C, can cause death of

insects within several hours. The 'Deglet Noor' variety of dates was found to be resistant to discoloration during short exposures of several hours at 55°C. Therefore, it was found that a combination of heat and carbon dioxide treatment is desirable to enhance mortality and emigration of the pests outside the treated fruits (UNEP, 2002). This combination treatment is used for disinfestations of organically grown dates. It has been also used in some countries for the disinfestations of 'Medjool' and 'Deglet Noor' (handled in 0.5 m^3 crates) marketed on branches (FAO, 2002).

5. Sulfuryl Fluoride. Sulfuryl fluoride (SF) is a non-flammable, non-corrosive to metals or equipments (Bell et al., 2004), odourless and colourless gas that boils at -55.2°C. This fumigant has been registered and used as a structural fumigant in EC and the USA and some other countries (UNEP, 2006). Sulfuryl fluoride can be used under reduced pressure (existing vacuum chambers) so that the exposure period can be reduced. Unfortunately, the SF producing company announced it would not sell its product in North Africa.

6. Ethyl Formate. Ethyl formate (EtF) is a volatile compound (liquid at normal ambient temperatures with boiling point of 54°C) and vaporizes at normal temperatures of stored products. It occurs naturally in a variety of products (0.05-1.0 mg/kg), including beef, cheese, rice (Desmarchelier, 1999), grapes and wine (Hiroyasu et al., 1972), and is generally recognized as a safe (GRAS) compound as it is known to break down into formic acid and ethanol. It has been demonstrated to have insecticidal properties (Rohitha et al., 1993) and leaves trace amounts of residues on treated products (Desmarchelier and Ren, 1999). Ethyl formate needs a 72-h minimum period of exposure for efficacy and is corrosive to unpainted metals.

The mode of action seems to be the inhibition of cytochrome C oxidase by the formic acid resulting of the hydrolysis of EF (Haritos and Dojchinov, 2003). In these conditions, gas tightness is not as important as with conventional fumigants because sorption takes place at a very high level.

This fumigant (EtF) is marketed under the trade name "VAPORMATE", a non-flammable product (Ryan et al 2002) which contains 16.7 wt% EtF dissolved in liquid CO_2 (equivalent to 11 vol.% EF in gaseous CO_2 when vaporized). Some experiments indicated that doses of 660 g/m^3 held for 4h or 420 g/m^3 held for 24h were needed to completely kill all stages of all insects.

Ethyl formate (Vapormate) has some advantages such as (1) short exposure time, (2) dose does not affect the quality of the treated products, (3) safe to use, (4) has no residual chemicals after the treatment and (5) no withholding periods after treatment (Vu and Ren, 2004).

7. Modified Atmosphere (CO_2 in Air). It is a definition that describes the modification of the composition of internal atmosphere in a closed area in order to increase the shelf life of the treated products. The modification process often depends on lowering the oxygen concentration by replacing it with carbon dioxide or nitrogen gas to inhibit the growth of all aerobic organisms (microorganisms, insects, etc.) and stop the oxidation reaction or keeping it at lower rates (Wikipedia, 2010). Very limited work has been carried out to determine the influence of modified atmospheres (MAs) on dates' quality.

El-Mohandes et al. (unpublished data) conducted a laboratory experiment to evaluate the use of CO_2 in air as MB alternatives for stored dates and its effect on controlling adults of *Oryzaephilus surinamensis* and *Tribolium confusum*. The variety used ('Sewi', semi dry) was at tamr stage with 22-24% moisture content. The experiment was carried out at 25±2°C and relative humidity of 55±5%. The effect of CO_2 was tested at different concentrations of 55, 65, 75 and 85% CO_2 in air for different exposure times of 6, 12, 18, 24, 36, and 48 hours for each CO_2 concentration. The control treatment was considered for comparisons. Three samples were taken after each exposure time for determination of mortality. The experiment was replicated three times.

The results indicated that in case of *Tribolium confusum*, 100% mortality was achieved after 36h at CO_2 concentrations of 75% whereas at CO_2 concentrations of 55, 65 and 85%, complete mortality (100%) was achieved after 48h exposure. In case of *Oryzaephilus surinamensis*, 100% mortality was achieved after 36h at CO_2 concentrations

of 55 and 75% whereas concentrations of 65 and 85% caused complete mortality after 48h exposure.

More research is needed to develop this technology to reduce the time needed for complete elimination of postharvest pests and hence allow adopting it at a commercial level.

8. Phosphine. Phosphine is a colorless, flammable and toxic gas at ambient temperature. Pure phosphine is odorless whereas technical grade phosphine has a highly unpleasant odor (garlic or decaying fish-like odor). The gas is slightly soluble in water and can be found in different presentations (tablets, pellets and bags) in form of magnesium or aluminum phosphide. In many countries, phosphine is used as MB alternative for fumigating various products against stored product pests (USDP, 1993; USEPA, 1989). Although effective, phosphine is very slow-acting (minimum of 5 days exposure) and does not force insects, like MB to emigrate from fruit (disinfestations). Phosphine efficacy had been proven against a broad range of pests, however, some reports showed that some pests' strains developed resistance to phosphine treatment (Nayak and Collins, 2000; Collins and Daglish, 2000).

In the dates industry, fumigations during the harvesting season must be rapid to enable a quick turnaround of the fumigation chambers with a minimum of one fumigation per day. Phosphine generated in pure form was found to be effective for high moisture dates, but a minimum fumigation period of 5 days is required to reach 100% mortality using the recommended dose (1.5-2 g gas/m^3). However, if treatment time of dates exceeds 72h, fermentations may take place and negatively affect the quality of treated fruits.

As any alternative, phosphine has some advantages and disadvantages. The main advantages are that (1) it is not reported as neither carcinogenic nor an ozone depleting substance and (2) easy to apply, (3) disperses rapidly inside the enclosure and no fans required, (4) cheap and airs off easily. The disadvantages are (1) not as effective as MB, (2) some insects built resistance to phosphine, (3) temperature dependent, not effective below 15°C and (4) highly explosive and corrosive to certain metals.

9. Phosphine and Carbon Dioxide (Combined Mixture). Cylinders-based formulations containing phosphine mixed with carbon dioxide (2%, balanced CO_2) have been developed recently but not yet widespread. The use of this form of phosphine shortened the time for effective disinfestations and left no solid residues after the fumigation (UNEP, 2002).

10. Phosphine and Modified Atmosphere (Combined Application). Phosphine tablets in form of aluminium phosphide can be used in combination with modified atmosphere (CO_2 in air) to reduce the time of exposure needed to achieve 100% mortality of postharvest pests compared to individual application. A laboratory experiment was carried out by El-Mohandes et al. (unpublished data) to evaluate the effect of different alternatives on (1) adult stage of *Oryzaephilus surinamensis* as well as adults and larvae stages of *Tribolium confusum* and (2) changes of chemical properties of treated date fruits. The determined chemical properties were free phenols, total sugars, reducing sugars and total amino acids. The alternatives tested were (1) recommended PH_3 dose (2 tablets/m^3), (2) PH_3 - half recommended dose (one tablet/m^3), (3) CO_2 (55, 65, 75 and 85%) + PH_3 (half PH_3 dose) and (4) control without treatment. The artificially infested dates with both insects were exposed to the tested treatments for 2, 3, and 6h. The experiment was carried out at 28±2°C and relative humidity of 60±5% where three samples were taken after each exposure time for determination of mortality and chemical analysis. The experiment was replicated three times.

The results indicated that application of CO_2 at concentrations of 55, 65, 75 and 85% with half dose of PH_3 caused 100% mortality, of all stages of tested insects, after 6h of exposure. The use of phosphine tablets alone either at half dose or recommended dose did not cause complete mortality to either stage of any insect when exposed to the same time periods. In terms of the chemical composition, the treatments slightly reduced content of free phenols, total sugars and reducing sugars content were increased whereas

total amino acids remained unchanged.

These results indicated that the use of CO_2 in combination with half a dose of PH_3 significantly shortened the time required to achieve complete mortality of infesting pests. Simultaneously, this treatment did not cause any noticeable changes in the tested chemical properties of treated dates.

11. Irradiation. Gamma rays, X-rays, accelerated electrons, or microwaves have been used experimentally to control insects. The Food and Agriculture Organization/ International Atomic Energy Agency (FAO/IAEA) promotes the use of radiation, recommending minimum doses of <4000 Gy for nematodes, <300 Gy for most insects, 100 Gy for *Diptera*, and 150-320 Gy for mites (Paul and Noel, 2002) as at such levels most fruits are not damaged (UNEP/TEAP, 2000).

In a laboratory experiment, El-Mohandes (unpublished data) studied the effect of irradiating a semi dry date variety ('Sewi') using gamma source at 0.5, 1.0, 2.0, 2.5 and 3.0 kGy on controlling *Tribolium confusum* (adults and larvae stages) as well as on total phenols and sugar content of treated samples. The results (unpublished data) indicated that the minimum irradiation dose that caused immediate 100% mortality of both stages was 2.5 kGy whereas exposing dates samples to 1.0 kGy caused 100% mortality after 192h post-irradiation. It was also noted that all irradiations doses significantly reduced free phenols content compared to the control samples, insignificantly reduced the total sugars and increased the reduced sugars.

These results indicated that radiations can be used as an effective treatment to control postharvest dates pests. However, there are some factors that limit the widespread use of radiation such as the public acceptance, the infrastructure required and to some extent the costs.

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Attraction of the Almond Moth, *Cadra cautella* (Walker), to Ready Made Tea or Coffee: an Alternation Method of Methyl Bromide in Storehouses of Date Fruit in Egypt

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Keywords: integrated pest management, baits, web building spider

Abstract

Cups of ready made tea or coffee were equally efficient in attracting and killing the almond moths of *Cadra cautella*, more than water, before egg laying. The ready made coffee was equal to the ready made tea in the attraction power. Baits of tea or coffee attracted significantly more moths than sweet water or tap water. The naturally existing cobweb spiders (*Theridiidae*) captured the moths during their flight over the tea or coffee baits mainly. The number of moths entangled inside spider webs was greater than those that sank in the tea or coffee solutions. Without the help of these baits, spider webs would have less catch numbers of the moths. So, it is generally recommended that these baits must be at the 4 corners of the store room to facilitate for the web-building spiders spinning their silk crossing the angle of the corner. Non-infested dates accompanying the infested dates continued without infestation in the presence of tea or coffee baits and the existing web-spinning spiders in the microhabitat over the baits. This technique could be used in conjunction with the natural existing web-building spiders that appear in each corner of the store room. The last may be considered as first record or discovery.

The application of tea or coffee trap baits with the natural existing cob-web spiders is an excellent example of functional date fruit integrated pest management inside storehouses. This may be a good safe replacement of methyl bromide as well as phosphine tablets.

INTRODUCTION

The almond moth *Cadra cautella* (Walker) is a major worldwide pest of stored foods. It occurs both in tropical and temperate regions and commonly attacks grains, nuts, dried fruits and a great variety of other stored products. It is a common, often serious pest of dried plant materials. It has been recorded from cereal grains and their products, dried fruit, nuts, oilseeds, pulses and cacao and also has been reported from dried maize (Arbogast and Chini, 2005). *Cadra* spp. (*C. cautella* and *C. calidella*) are the most important insect pests that start in the field and continue in storehouses. In this respect, Sayed and El-deeb (1996) indicated that sex pheromones played a significant role inside the storehouse and not in the field. *Cadra* (*Ephestia*) *kuehniella* Zell is the most important pest arriving in the Al Hassa factory from the field in Saudi Arabia (Al Jabar, 2003). Satisfactory control was achieved by covering date bunches during the 1st half of July (Essa, 2003).

Infestation mostly comes from the field as eggs or larvae (and maybe pupae), then emerge and attack new non-infested dates in storehouses. Also, the adult moths may come from the field and search to locate the non-infested dates in the storehouses. Killing these moths before egg deposition with non-insecticides is a good safe tool to stop continuing infestation or avoid starting the infestation in storehouses. Before harvest, to minimize or prevent infestation coming from the field to storehouses spraying date trees with safe friendly chemicals in rotation is essential.

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Due to residue toxicity, the Ministry of Agriculture (MOA) in Egypt banned all conventional insecticides from use on dates. They allowed only bio-insecticides or organic or safe products. Current available bio-insecticides are slow acting and do not satisfy farmers need (Sayed et al., 2001). Farmers started to use Bio-products (e.g., *Bacillus thuringiensis*) but due to the very poor performance under the hot sun, they stopped using these products. Spinosad acts quickly and has a speed of kill comparable to most synthetic insecticides. It acts significantly faster than slow acting products like *Bacillus*, *Beauvaria* and other traditional biologicals (Bret et al., 1997).

Spinosad was considered as organic in the USA and EU in 2002 and 2008, respectively. It came as fermentation from the soil bacteria *Saccharopolyspora spinosa* Mertz and Yao (1990). One key pest management strategy is the rotation of different mode of action to ensure that continual selectivity for resistance does not occur and the possibility of pest resistance is avoided or at least significantly delayed (Thompson et al., 1997; Temerak, 2003). Application of Tracer at 20 ml in rotation with Runner 24 SC at 15 ml/100L is an excellent example of a functional date palm integrated pest management program (Temerak and Sayed, 2007). Based on Temerak and Sayed (2007), spray before harvest, as application of Tracer 24 (spinosad) SC at 20 ml/100 L at end of June and by Runner 24 SC (Methoxfenozide) at 15 ml/100 L after 3 weeks interval in the field will help to have at least less infestation in storehouses later. The availability of a novel chemical group as spinosad with a new mode of action that is different from insecticides in current use, is an asset to insecticide resistance management programs (Kranthi et al., 2000). Spinosad was not easily affected by the existing resistance mechanism (Temerak, 2003).

Methyl bromide is frequently used as a fumigant for disinfestations of insects in stored agricultural commodities such as nuts, cereals, dried vegetables and fruits. Methyl bromide has carcinogenic effects. Therefore, the application of methyl bromide as a fumigant is prohibited in many countries. Methyl bromide has been identified as a chemical that depletes the earth's ozone layer, and thus its use is being phased out. The United States is in the process of implementing a methyl bromide use reduction. There is no known single alternative fumigant, chemical or other technology that can readily substitute for methyl bromide in effectiveness, low cost, ease of use, and wide availability. Phosphine, a common grain dried fruit fumigant, is usually applied as tablets. The alumonium phoshpide products used as tablets are also highly hazardous. No considerable work was available in the literature dealing with tea or coffee baits. The current work is applying different safe baits to combat *Cadra* spp. in storehouses.

MATERIALS AND METHODS

Baits (Treatments)

1. Ready made cup of tea (2.25 g) using 3 spoons of sugar (8.75 g) in 200 ml of water was made and left to cool at room temperature.
2. Ready made cup of coffee (2.5 g) using 3 spoons of sugar (8.75 g) for 200 ml water was made and left to cool.
3. Cup of sweet water using 3 spoons of sugars (8.75 g) in 200 ml of water.
4. Cup of tap water, 200 ml.

Treatments

- 1st trial = tea versus water (distance between the 2 baits is 30 cm).
- 2nd trial = coffee versus water (distance between the 2 baits is 30 cm).
- 3rd trial = sweet water versus tap water (distance between the 2 baits is 30 cm).
- 4th trial = tap water.

Every treatment is replicated as 4 times (one trial in each room corner). Four dark rooms served as storehouses in the New Valley (west Egypt).

Each of the above trials was accompanied by 30 kg of old infested dates (harbouring full grown larvae and pupae) + another 20 kg clean without infestation of the

same variety ('Seidi'). The last were placed in the centre of the room in two open separate carton boxes. The room for each trial measured 3×4 m. Trials were made under 34°C and relative humidity 65%. Containers were placed on a small table 1 m high at each corner of the dark room.

Measurements

Moths that had sunk in each container were recorded every week, then a new same treatment was replaced. Moths entangled in the natural web building spiders area established over the bait were counted and removed by forceps on a weekly basis.

A sample of 10 date fruit was taken from the non-infested dates accompanying the old infested dates, for each treatment weekly (40 fruit ones/each trial/4 weeks).

Statistical analysis was done using a complete block randomised design. LSD was presented to compare means.

RESULT AND DISCUSSION

Table 1 shows the attracted numbers of almond moths to the baits. Cups (baits) of ready made tea or coffee were equally significantly efficient in attracting and killing the almond moths of *Cadra* spp., more than water, before egg laying. The ready made coffee was equal to the ready made tea in attraction power. The baits of tea or coffee attracted significantly more moths than sweet water or tap water. Sweet water attracted significantly more numbers of moths than tap water without sugar. We believe that the brown color of tea or coffee plays a significant major part in this attraction, more than sugar.

Table 2 shows the number of moths entangled in the spider web at each corner (total of 4 corners/room). One day after setting up the station of baits, the web-building spider start spinning webs in each corner after the emergence of adults, on the top of bait containers. Cobweb spiders (*Theridiidae*) built irregular cobwebs at each corner of the room at different height levels (25-35 cm). Spiders built the webs in these protected places. Typically, they have a spherical abdomen. The house spider frequently lives in buildings, typically locating its web in the corners of rooms or in the angles of windows. Cobweb spiders are common inhabitants of dark corners around the home. The family (*Theridiidae*) is also called comb-footed spider because they have a 'comb' on their last pair of legs. The comb is a series of serrated spines which they use to comb out the silk from the spinnerets (Knoflach, 2004). It is known also as the tangle-web spiders are a large family (over 2200 species in over 100 genera) of three-dimensional space-web-builders found throughout the world (Agnarsson, 2004).

Considerable numbers of moths have been entangled above tea or coffee and a significantly greater number than those of sweet water or tap water. The number of captured moths was greater inside the spider webs than those that sank in the tea or coffee solution baits. Without the help of these baits, spider webs would have less catch numbers. The naturally existing cobweb spiders captured significantly greater moths in the microhabitat when they fly over the tea or coffee baits than over sweet or normal water.

So, it is generally recommended that these baits must be at the 4 corners of the room to facilitate the web-building spiders spinning their silk crossing the angle of the corner.

Table 3 is a combination of Table 1 and Table 2. It shows the combined actions of baits plus webs expressed as number of killed moths. The joint actions indicated that tea versus water and webs was equal with coffee versus water and webs. The sweet water versus water plus webs significantly counted a greater number of moths than water and webs.

Table 4 indicates the number of infested fruit having signs of infestation or alive larva of the almond moth in the 20 kg (not infested before) accompanying the 30 kg with old infestation. Non-infested dates accompanying the infested dates continued without infestation in the presence of tea or coffee baits and the existing natural web-building

spiders only during 4 weeks. The last means that attraction took place before egg-laying. However, tap water showed 15% infestation.

Table 5 indicates all statistical analysis for Tables 1, 2, 3, and 4.

CONCLUSION

Applying a rotation program of Tracer then runner (Temerak and Sayed, 2007) in the field, followed by these tea or coffee baits in the presence of natural existing cob-web spiders in storehouses, is an excellent example of functional date fruit integrated pest management inside storehouses. So, it is generally recommended that these baits must be at the 4 corners of the room to facilitate the web-building spiders spinning their silk crossing the angle-corner. It is generally assumed, in case field control did not take place, that sticky pheromone traps can go in conjunction with these baits and the natural web-building spider's presence. Moreover, releasing parasitoid or predators in a storehouse to combat this insect pest is not a wise idea. These bio-tools are density dependant and no consumer likes to eat dates with any infestation.

These result may be considered as the first record or discovery as a good safe solution to replace methyl bromide and phosphine tablets from the use in storehouses. Additional research should be done to improve the above technique.

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Tables

Table 1. Number of almond moths, *Cadra cautella* sunk inside baits (total of 4 replicates).

Weeks	Trial 1		Trial 2		Trial 3		Trial 4
	Tea	Water	Coffee	Water	Sweet water	Tap water	Tap water only
1st week	7	1	6	0	2	0	1
2nd week	4	0	4	1	1	1	0
3rd week	3	0	3	1	1	1	1
4th week	2	1	2	0	2	1	1
Total	16	2	15	2	6	3	3

Table 2. Number of almond moths *Cadra cautella* entangled in the spider web (total of 4 corners).

Weeks	Trial 1 Tea vs. water	Trial 2 Coffee vs. water	Trial 3 Sweet water vs. water	Trial 4 Tap water only
1st week	2	3	0	0
2nd week	3	4	1	0
3rd week	6	5	1	1
4th week	9	9	3	2
Total	20	21	5	3

Table 3. Number of almond moths *Cadra cautella* as a total of the combined action of the Baits plus the webs (total of Tables 1 and 2).

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Weeks	Trail 1	Trial 2	Trial 3	Trial 4
1st week	10	9	2	2
2nd week	7	9	3	0
3rd week	9	9	3	2
4th week	12	11	6	2
Total	38	38	14	6

Table 4. Number of fruit having alive larva or sign of infestation of *Cadra cautella* in the non-infested dates accompanying the old infested date fruit.

Weeks	Trial 1 Tea vs water	Trial 2 Coffee vs water	Trial 3 Sweet water vs water	Trial 4 Tap water only
1st week	0	0	0	0
2nd week	0	0	0	1
3rd week	0	0	1	2
4th week	0	0	2	3
Total	0	0	3	6

Table 5. Statistical analysis summary of Tables 1, 2, 3 and 4.

Treatments	Mean number of almond moths			Mean number of date fruit having infestation
	Sank inside baits	Entangled In webs	As combined of baits + webs	
Tea vs. water (Trial 1)	1.00[a]	1.25[a]	2.38[a]	0.00[c]
Coffee vs. water (Trial 2)	0.94[a]	1.31[a]	2.38[a]	0.00[c]
Sweet water. vs. water (Trial 3)	0.38[b]	0.31[b]	0.88[b]	0.19[b]
Water (Trial 4)	0.19[c]	0.19[b]	0.38[c]	0.38[a]
F value	15.75	40.71	51.00	4.57
LSD at 0.05	0.18	0.30	0.34	0.16

Mean values of treatments over all weeks.
Figures followed by the same letter are not significantly different.

Disinfestation of Dates Using Electron Beams in Comparison with Other Treatments

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Abstract

In this study electron-beams were applied to disinfestate dates through using 5 different energy/doses and comparing disinfestation efficacy such as insect mortality and hatches with microwave, steaming and fumigation by phostoxin. The percentage of mortality was 100% for all electron-beam treatments, also hatches were less (0-1) compared to microwave, steaming and fumigation. The treated dates' quality was assessed by measuring moisture content, water activity, color, total viable count (TVC), yeast and mould count (YMC) and antioxidant capacity. There was no difference in moisture content between control and other treated samples except for steaming and fumigation, whereas moisture increased to 14.59 and 15.07 g/100 g respectively. Alike to the changes pattern of the moisture content, the steam treatments as well as the fumigated samples reveal a slight increase in the water activity. Color lightness was lowest in the steaming sample (20.36); conversely, it remains almost unchanged through other treatments. Electron beams (1.5 MeV/1.0 kGy and 1.5 MeV/2.0 kGy) had the lowest TVC (1.65 and 1.70 log cfus/g), and also YMC was low in samples treated by electron beams in comparison with other treatments. There were no significant differences in antioxidant capacity between all treatments, except for the sample treated by electron beams 1.5 MeV/ 2.0 kGy, antioxidant capacity was reduced to 232 μmol/g. EPR free radicals generation (g-value) measurements through alanine dosimetry were 1×10^{-8} mol/J lower than the alanine (3×10^{-7} mol/J).

INTRODUCTION

In the presence of international standards that reject any lot containing even residues of dead insects; dates infestation during storage becomes increasingly a serious obstacle that faces the development of the dates sector. Thus, an efficient disinfestation appears vital to the producers as well as processors. In addition, complementary improvements, particularly those connected to the postharvest practices, are necessary to obtain a high quality infestation free produce. Methyl bromide (CH_3Br) is still the main and the most successful fumigant of date's bulks. It is still in use; nevertheless, methyl bromide will be banned from use as a fumigant as it is a depleting substance of the ozone layer according to the Montreal Protocol. Thus, alternatives seeking should begin seriously. Since a while, phosphine (PH_3) that generates through the reaction of aluminum (AlP) or magnesium phosphide (Mg_3P_2) with humidity traces, has been applied extensively to fumigate dates. However, several re-infestations of the fumigated dates have been observed while doubt arose about the efficiency of phosphine. Assessment studies of the fumigation of dates by phosphine shows that three widespread storage insects such as *Plodia interpunctella*, *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* have been 100% terminated (Al-Abid and Schmitt, 2006). Nevertheless, these results have been achieved through maintaining precise experimental conditions that might not be applicable for common farmers. Several studies reported the mortal effect of electron beams on common pests (Salimov et al., 2000; Cleghorn et al., 2001; Todoriki et al., 2006). Previous studies found electron-beam irradiation doses 0.5-1.0 kGy maintained the quality of mangoes and had no adverse effect on physico-chemical and nutritional

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properties of apricot (Moreno et al., 2006; Egea et al., 2005). In view of that, the electron beam technique has been chosen as alternative continuous disinfestation technique that does not cause pollution to the environment. To assess the treatments by the electron beam, the outcomes have been compared with further available disinfestation techniques such as phosphine fumigation, microwaves as well as heat treatment through steaming. The comparison included effects of these treatments on some relevant date quality criteria like moisture content, water activity, color, microbial load and antioxidant capacity.

MATERIALS AND METHODS

Plant Materials

Dried dates ('Fard') obtained from a local farm in Nizwa, Oman, were used in this study. The dates infested by *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* were incubated at 30-40°C for 3 months to boost insect reproduction. The date samples (100 g) were packaged in polyethylene bags, sealed using an Ankom heat sealer, and stored at room temperature for treatments afterwards as well as analysis. The samples after each disinfestation treatment were packed in aseptic bags and sealed for quality analysis.

Disinfestation Treatments

1. Electron Beams. Dates were placed in a single layer in cardboard on a conveyor and exposed to electron beam 0.5, 1.0, or 2.0 kGy doses using 0.6, 1.0, or 1.5 MeV linear accelerator with single beam fixture. The electron beam treatment was carried out at the Fraunhofer Institute for Electron Beam and Plasma Technology, Dresden, Germany. The control was the non-treated dates.
2. Microwave. A household microwave with output power of 750 watt and frequency at 2.45 GHz (SHARP, Sharp Corporation, Japan) was used to disinfect dates for 60 s according to Nelson et al. (1974).
3. Steaming. One layer of dates was placed in a sieve, steamed for 10 min using steam produced by a boiling water bath according to MAF (2003).
4. Fumigation. A date sample was placed in a 1 m^3 sealed box and fumigated by 3 g phostoxin tablet (Detia Degesch Phostoxin) using the dose of 1 tablet/m^3 and kept for 72h before ventilation according to the producing company leaflet.

Quality Analysis

1. Moisture, Water Activity and Color. The moisture content of dates was determined by the AOAC official method of analysis of moisture in fruits by oven drying (AOAC 1995, No. 934.06). Water activity was measured using an AW SPRINT (Novasina, Swiss made) instrument. The dates' color (Hunter lab) was determined using a Spectrophotometer NF333 (Nippon Denshoku IND.co Ltd, Japan).
2. Insect Mortality and Hatch Count. To figure out the mortality through treatments, each sample of triplicate that contained 10 fruits was used to count the live and dead insects manually by using magnifying lenses. The counts of deceased insects have been reported as percentage of mortality. Subsequently, the same samples, free from live and dead insects, were incubated at 30-40°C for 45 days to count the hatches.
3. Microbial Analysis. Total viable count (TVC) was carried out through blending 10 g of dates sample with a 90 ml Ringer solution (1/4 strength) for 10 min in a sterilized blender. Serial dilutions were made from this homogenate that were used to inoculate culture plates. The developing colonies through following incubation at 30°C for 48h were reported as colony forming units (CFU). Plates containing more than 300 colonies and less than 30 were neglected. Colonies were calculated as log CFU/g. Yeast and mould count (YMC) was carried out according to Omani Standard no. 117 (1986) with potato dextrose as culture media incubated at 21°C for 5 days.
4. Antioxidant Capacity. Antioxidant capacity in dates was determined using the modified Oxygen Radical Absorbance Capacity (ORAC) assay (Huang et al., 2002; Prior

et al., 2003) using a Shimadzu RF-1501 spectrofluorometer (Shimadzu corporation, Japan).

5. Statistical Analysis. Results were expressed as mean value ± SD (n=3) on a fresh weight basis. Statistical significance (t-test: 2-sample assuming equal variances) performed using the Microsoft Excel Data Analysis (Microsoft Corp., Redmond, Wash., USA). Differences at $P<0.05$ were considered to be significant.

Measurement of Electron Beam Induced Radicals in Dates

Continuous wave electron paramagnetic resonance (EPR) spectroscopy has been used to determine the concentration of free radicals, which is proportional to the absorbed dose and the mass of the sample. A compact EPR spectrometer dosiscope DS 100 has been used, which includes a 9.6 GHz oscillator and a reference arm to achieve a high sensitivity in a wide microwave power range. The dosiscope DS 100 was connected to a PC via RS 232 interface. A special program for data acquisitions and evaluation accomplish the system and ensures the calibration of the spectrometer for each run using a reference sample. The EPR-measurements have been carried out in the Frauenhofer Institute/Germany for different date skins compared with the surface of alanine dosimeters. Both have been treated with 2 kGy as highest applied dose.

RESULTS AND DISCUSSION

The moisture content of treated date samples as shown in Table 1, ranged between 10.53 to 15.07 g/100 g. It shows minor differences between the electron beam and the microwave treatment compared with the control, whereas samples treated by steaming and fumigation show an increase in the moisture content. This could be due to the steam and the ambient humidity of the sealed fumigation chamber. However, the acquired moisture was still not enough to promote the microbial growth in the dates. As Table 1 reveals, water activity ranged between 0.49-0.56. Alike to the changes mode of the moisture content, the steam treatments as well as the fumigated samples reveal a slight increase in the water activity value. Nevertheless, the measured a_w were far below to endorse the microbial growth as well as enzyme activation.

Color lightness (*L) of dates is regarded as a quality criterion since dates, as reduced sugar rich fruits, are vulnerable to heat as well as radiation. The steaming and electron beam 1.5/0.5 MeV/kGy treatments show the lowest (*L) values compared to others (Table 2). Obviously, these treatments enhance the rate of the non-enzymatic browning. Previous study reveals that the decrease in the (*L) value of irradiated cantaloupe was proportional to the increase of electron beam doses from 0.7 to 1.4 kGy (Palekar et al., 2006). On the other hand, no change in color has been reported as blueberries were irradiated by an electron beam of 1 kGy (Miller et al., 1995).

The mortality of the live insects was 100% through all treatments (Table 3). The hatch was nil in the irradiated sample with dose of 0.6 MeV /2.0 kGy and 1.5 MeV/ 0.5 kGy. Nonetheless, the percentage of mortality for disinfestation treatments is shown in Table 3. Microwave, steaming and fumigation had less effect on the hatch, as they were 4, 5 and 6, respectively. The hatch count took place after an incubation period of 45 days and ambient temperature of 30-40°C. These results reveal that proper doses of electron beams are able to penetrate the fruit flesh to terminate the metamorphosis of the storage beetles including the eggs and hence can be a remedy for re-infestation.

Electron beams significantly reduced the total viable count (TVC) of microbes to the lowest level clearly more than the other treatments (Table 3). The effect of electron beam on TVC increased with the increase of doses. TVC was 2.48 log cfus/g after treating with 0.6/2.0 MeV/kGy and reduced to 1.70 log cfus/g with 1.5/2.0 MeV/kGy. Steaming (1.90 log cfus/g) had better eliminating effect on TVC than microwave (2.81 log cfus/g) and fumigation (2.84 log cfus/g). Yeasts and moulds counts (YMC) results (Table 3) demonstrate again the superiority of the electron beam treatments in reducing the YMC. The effect on YMC became ascending due to doses increase from 3.00 log cfus/g at 0.6/2.0 MeV/kGy to zero at 1.5/2.0 MeV/kGy. Microwave, steaming

and fumigation had less effect on YMC, as they were 3.00, 2.00 and 1.30 log cfus/g, respectively. Nevertheless, all treatments reduced the microbial load to acceptable limits that food controllers permit. The microbial reference criteria for dried fruits has maximum value of 6 log cfus/g for aerobic plate count and 3 log cfus/g for yeasts and moulds, above these values samples are unacceptable (FDA,1995). Previous work on the effect of electron beams on different washed cantaloupe revealed that the microbial load reduction was proportional to the irradiation dose (Palekar et al., 2004). A further study showed that electron beams levels above 0.5 to 5.2 kGy reduced total plate counts, yeast and mould, and psychrotrophic counts to below detectable levels and thus prevented microbial-induced browning in mushroom (Koorapati et al., 2004).

To indicate a likely consequence of reactive oxygen species stress generated by the irradiation, the antioxidant capacities have been measured. Moreover, antioxidant capacity is regarded nowadays as a significant hidden attribute; therefore any treatment of dates should maintain such quality norm. The results show an increase in the antioxidant capacity of most of the treated samples except the 1.5/2.0 MeV/kGy treatment, which shows a decrease to 232 μmol/g (Table.4). Increases of antioxidant capacity suggest the development of products with enhanced antioxidant capacity through different mechanisms of action. Formation of compounds with increased ability to donate electrons or transfer hydrogen atoms is reflected in the ORAC assay (Huang et al., 2005). Electron-beam treated blueberries show a significant increase (10 to 20%) in total phenolics and tannins content compared to the control (Moreno et al., 2005). Flavonoids can polymerize under adequate conditions. It has been proposed that these polymers may be more potent antioxidants than their monomers (Senter et al., 1978; Hagerman et al., 1998). Procyanidin polymer of varying degree, which is believed to be a significant source of antioxidant capacity, has been isolated and chemically characterized in the date skin (AlAbid, 2003). The polymerization takes place along with fruit maturation and storage. The oxidation of flavonoids into polymers of varying degrees of polymerization has been reported in pecan kernels stored for 7 days at 70°C (Senter et al., 1978). In the same study, authors reported that this oxidation is progressive, which suggests an increase in degree of 40 polymerization over time. Recent study unveiled that irradiation by electron beams decreased the total phenol content and condensed tannins in the pecan kernels with no major detrimental effects in antioxidant capacity (Villarreal-Lozoya et al., 2009). On the other hand, electron-beam irradiation boosts the antioxidant capacity in the citrus pomaces (Kim et al., 2008). The microwave as well as steaming treatment improve the antioxidant capacity and with less extent the fumigation (Table 4). Due to the developed heat through those treatments incorporated with other supporting factors such as humidity and pH the antioxidants may release from the complexes where they are bounded. So, the linkages between *p*-coumaric acid and lignin and between ferulic acid and arabinoxylans could be cleaved at high temperature (Maillard et al., 1995). Hydrolysable tannins produce glucose and phenolic acids such as gallic and hexahydroxydiphenic acids (Shahidi et al., 1995).

As additional measurement to verify the generation of free radicals during the radiation, alanine dosimetry has been applied. The amino acid alanine is used to control free radicals produced by ionizing radiation (gamma rays, electrons, neutrons, charged particles). This technique is called alanine dosimetry, which is the internationally accepted method for dose measurements in different radiation fields. Through the yield of radical generation in alanine (so called g-value), which was about 3×10^{-7} mol/J, the calculation of the g-value of date skin has been enabled. The EPR-measurements have been carried out on different date skins in comparison to the surface of alanine dosimeters. Both have been treated with 2 kGy. The results disclose a g-value of about 1×10^{-8} mol/J, below the one of the alanine. Figure 1 clearly demonstrates the fluctuation of the alanine relative intensity/mm^2 value above and below the zero line due to the change of the magnetic field, whereas the date skin remains stable close to the zero limits.

According to above results the irradiation by electron-beam should be considered as an efficient and safe technique to disinfest dates.

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Tables

Table 1. Moisture content, water activity and color of treated dates.

Treatments	Control	Electron beams (MeV/kGy)					Microwave	Steaming	Fumigation
		0.6/2.0	1.0/2.0	1.5/0.5	1.5/1.0	1.5/2.0			
Moisture (g/100g)	10.97 ± 0.11[a]	10.53 ± 0.19[a]	10.74 ± 0.11[a]	10.77 ± 0.22[a]	10.83 ± 0.08[a]	10.66 ± 0.14[a]	11.60 ± 0.03[b]	14.59 ± 0.42[c]	15.07 ± 0.43[c]
Water activity	0.50 ± 0.011[a]	0.50 ± 0.002[a]	0.50 ± 0.002[a]	0.51 ± 0.002[a]	0.51 ± 0.004[a]	0.50 ± 0.001[a]	0.49 ± 0.005[a]	0.56 ± 0.016[b]	0.52 ± 0.006[a]
Color (L)	28.57 ± 1.1[a]	27.37 ± 1.4[a]	26.89 ± 0.84[a]	22.88 ± 0.47[b]	29.35 ± 0.87[a]	25.34 ± 0.84[a]	26.42 ± 0.28[a]	20.36 ± 0.95[c]	27.31 ± 1.2[a]

Data are expressed as mean ± SD (n=3). Means ± SD followed by the same letter within a row are not significantly different (P>0.05).

Table 2. Percentage of mortality and hatched eggs count of treated dates.

Treatments	Control	Electron beams (MeV/kGy)					Microwave	Steaming	Fumigation
		0.6/2.0	1.0/2.0	1.5/0.5	1.5/1.0	1.5/2.0			
% Mortality	0 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Hatch count	23 ± 2.0	0 ± 0	1 ± 0.58	0 ± 0	1 ± 0.58	1 ± 0.58	4 ± 0.58	5 ± 1.15	6 ± 1.15

Triplicate samples each contain 10 fruits were used to count the live and dead insects visually, data are expressed as mean ± SD.

Table 3. Total viable count (TVC) and yeast and mould count (YMC) of treated dates.

Treatments	Control	Electron beams (MeV/kGy)					Microwave	Steaming	Fumigation
		0.6/2.0	1.0/2.0	1.5/0.5	1.5/1.0	1.5/2.0			
TVC (log cfus/g)	3.16[a]	2.48[b]	2.88[c]	2.88[c]	1.65[d]	1.70[e]	2.81[f]	1.90[g]	2.84[h]
YMC (log cfus/g)	3.30[a]	3.00[b]	1[c]	0[d]	1[c]	0[d]	3.00[b]	2.00[e]	1.30[f]

Data are expressed as mean (n=3), if followed by the same letter, within a row, are not significantly different (P>0.05).

Table 4. Antioxidant capacity of treated dates.

Treatments	Control	Electron beams (MeV/kGy)					Microwave	Steaming	Fumigation
		0.6/2.0	1.0/2.0	1.5/0.5	1.5/1.0	1.5/2.0			
Antioxidants (µmol/g)	289 ± 9^{a}	301 ± 12^{a}	321 ± 16^{b}	379 ± 13^{c}	295 ± 2^{a}	232 ± 13^{d}	375 ± 6^{c}	365 ± 12^{c}	291 ± 5^{a}

Data are expressed as mean ± SD (n=3) on a wet weight basis. Means ± SD followed by the same letter, within a row are not significantly different (P>0.05).

Figures

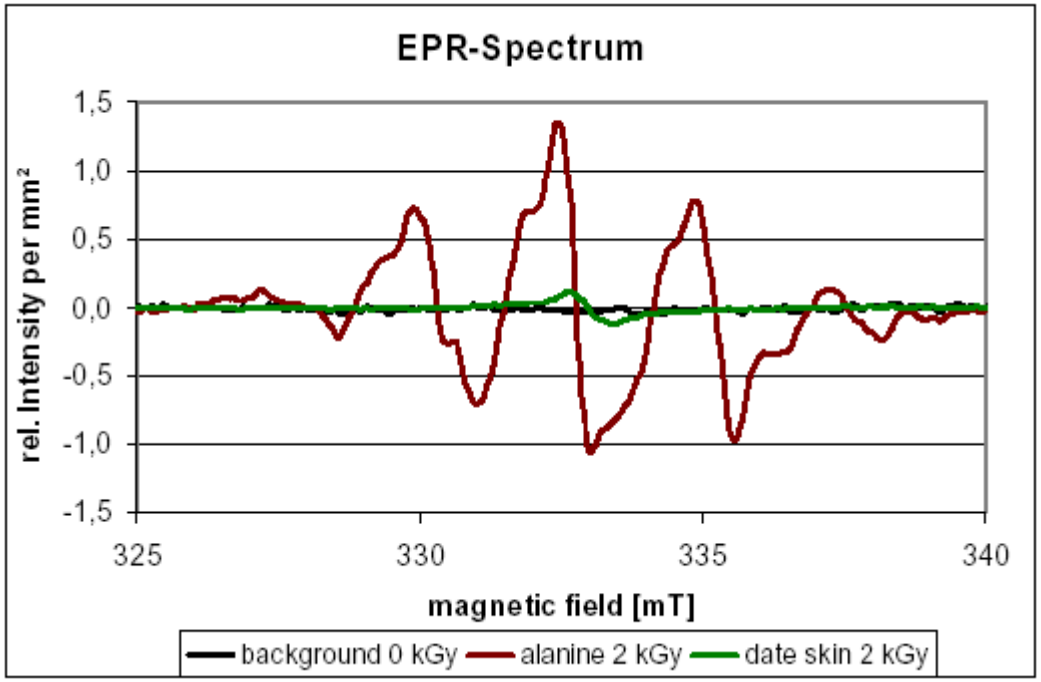

Fig 1. EPR-spectrum of date skin in comparison to alanine dosimeters.

Implementation of a Demonstration Project on Alternatives to Methyl Bromide for the Treatment of High Moisture Dates (Algeria and Tunisia): the UNIDO's Experience

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Abstract

In Decision XV/12, the Parties to the Montreal Protocol recognize the risk of potential non-compliance vis a vis the agreed phase-out schedule for those A5 countries that rely on the use of Methyl Bromide (MB) to stabilize and disinfest high moisture dates at time of harvest. Indeed, up to now, the Methyl Bromide Technical Option Committee of TEAP has not been able to identify feasible alternatives to replace the use of this fumigant in the specific sector of high-moisture dates (see 2006 MBTOC Assessment report, 2008 TEAP progress report).

UNIDO took the initiative to respond to Decision XV/12 and a project proposal was designed to demonstrate the feasibility of alternatives to MB for the post-harvest treatment of high moisture dates. The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available in Algeria and Tunisia as well as in similar conditions and circumstances.

A preliminary investigation of potential pest control techniques was launched. Five alternatives were tested in 2008 in a small-scale laboratory test in France, using 'Deglet Nour' from Biskra, Algeria.

The tests have been conducted with limited replicability and with limited control on efficacy and effect on fruit quality. Furthermore, the environmental conditions were different from the conditions that might be expected in north African countries at the time of treatment. Despite the fact that the test provided interesting technical information, it should be considered of limited scientific value.

UNIDO presented the results of that preliminary investigation holding a technical workshop on the replacement of methyl bromide for the disinfestation of high moisture dates in Vienna (16-17 April 2009).

For the time being, two alternatives have been considered promising for the full scale tests: phosphine without ammonia and high temperatures.

The full scale demonstration will be designed to assess the efficacy of the two alternatives on all the live stages of pests (in particular on eggs of *Lepidoptera*) and to assess the effect on fruit quality.

Experience has shown that full involvement of local experts and expertise is needed for the successful preparation, design and implementation of the activities.

BACKGROUND

In Decision XV/12, the Parties to the Montreal Protocol recognize the risk of potential non-compliance vis a vis the agreed phase-out schedule for those A5 countries that rely on the use of Methyl Bromide (MB) to stabilize and disinfest high moisture dates at time of harvest. Indeed, up to now, the Methyl Bromide Technical Option Committee of TEAP has not been able to identify feasible alternatives to replace the use of this fumigant in the specific sector of high-moisture dates (see 2006 MBTOC Assessment report, 2008 TEAP progress report).

UNIDO took the initiative to respond to Decision XV/12 and a project proposal was designed to demonstrate the feasibility of alternatives to MB for the post-harvest

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treatment of high moisture dates. The objective of the project is to demonstrate whether alternatives to MB for the treatment of high moisture dates are technically and economically available in Algeria and Tunisia as well as in similar conditions and circumstances.

PROJECT IMPLEMENTATION

A preliminary investigation of potential pest control techniques was launched. Five alternatives were tested in 2008 in a small-scale laboratory test in France, using 'Deglet Nour' from Biskra, Algeria.

The tests have been conducted with limited replicability and with limited control on efficacy and effect on fruit quality. Furthermore, the environmental conditions were different from the conditions that might be expected in North African countries at the time of treatment. Despite the fact that the test provided interesting technical information, it should be considered of limited scientific value.

UNIDO presented the results of that preliminary investigation holding a technical workshop on the replacement of methyl bromide for the disinfestation of high moisture dates in Vienna (16-17 April, 2009).

First of all, the following definition for "high moisture dates" was proposed: dates at high water content are dates of the 'Deglet Nour' variety, with moisture content from 30 to 40% (compared to the wet weight). The colour of such dates is light and somewhat transparent. These dates are marketed still attached to small branches. The relative humidity in equilibrium with these high-moisture dates allows the rapid development of yeasts resulting in fermentation, if the dates are either stored or fumigated in gas tight conditions. Gas tightness sufficient for fermentation can also occur in consumer packaging.

Secondly, experience (including the test in France) and research on the following five potential MB alternatives have been presented and discussed: heat treatment, ethyl formate, phosphine, modified atmospheres and sulphuryl fluoride. The main conclusions were:

- Experience conducted demonstrated that controlled atmosphere (exposure time of 7 days and an average temperature of 12.5°C) facilitated fermentation, resulting in a high loss of the fruit quality.
- Sulphuryl fluoride and ethyl formate were both promising in that they controlled pests (on larvae and pupae), they can be used in the existing vacuum chambers, and they only require short exposure times. Unfortunately, due to lack of registration and lack of available suppliers, these two potential alternatives had to be eliminated for use in north African countries. Furthermore, ethyl formate is not registered as an insecticide in the EU, the principal market of north African dates and no company seems eager to register it. So, although ethyl formate has been registered in Israel and found effective for the control of pests in high moisture dates, it is not available to north African date producers, conditioners and/or exporters. Therefore, these two alternatives will not be studied further in the next tests under the project.
- Phosphine generated in pure form (and not from formulations containing ammonia), was found to be technically effective for high moisture dates on branches, although using this technique resulted in the need to change treatment logistics. Managing treatment time when using this technique is important. If the treatment time exceeds 72h, fermentation can result. Thus further work is needed to clarify this method. A phosphine product formulated as gas mixed with CO_2 was considered even more effective. Phosphine generators producing a mixture of PH_3/CO_2 at a concentration 2/98% are available.
- Heat treatment, (50°C for 2h with a 2-h come-up time) was previously found to be effective for other date varieties and of drier moisture contents ('Medjoul'). Recent preliminary studies in Israel found the same method to be quite promising for high moisture dates on branches. Work on this technique is ongoing. If not done properly, heat can produce a non-desirable effect of cracking and pasty texture. Thus further work

is also needed to clarify this method for high moisture dates.

A date producer reported that deep freezing (-25°C) is currently used for the treatment of fresh 'Deglet Nour' in branches for the organic market. This treatment requires a very high investment and high operating costs so it was determined it could not be considered as an alternative for the entire production of fresh and high-moisture dates. Furthermore, experience on date pasteurization was presented and considered with some technical interest.

CONCLUSIONS AND NEXT STEPS

For the time being, two alternatives have been considered promising for the full scale tests: phosphine without ammonia and high temperatures.

The full scale demonstration will be designed to assess the efficacy of the two alternatives on all the live stages of pests (in particular on eggs of *Lepidoptera*) and to assess the effect on fruit quality.

Experience has shown that full involvement of local experts and expertise is needed for the successful preparation, design and implementation of the activities.

ACKNOWLEDGEMENTS

The Regional Demonstration Project on alternatives to the use of methyl bromide for treatment of high moisture dates is financed by the Multilateral Fund for the Implementation of the Montreal Protocol.

Evaluation of the Nutritional Value of Functional Yoghurt Resulting from a Combination of Date Palm Syrup and Skim Milk

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Keywords: Yoghurt, date palm, minerals, folate, antioxidant activity

Abstract

The development of fermented products with a new flavor and health benefits helps the dairy industry increase its sales. Date fruit is one important source of supplying minerals and vitamin elements with acceptable taste. The objective of this study was to use date palm syrup as a part of water (v/v) used in reconstituting skim milk powder in processing yoghurt with 14% total solids. Physical properties such as sensory characteristics and apparent viscosity were evaluated. To evaluate the nutritional value of yoghurt, antioxidant values were monitored during storage and the sample which recorded the highest values would determine its chemical composition. In addition, some micronutrients (HCl-soluble minerals) and (folate and C vitamins) were compared to plain yoghurt. Results showed that yoghurt enriched with 10% date syrup had a significant sweetness, recorded the highest antioxidant values, was higher in HCl-soluble minerals and folate concentration compared to plain yoghurt. It could be concluded that numerous health benefits beyond its nutritional value have been associated with consuming yoghurt enriched with 10% date palm syrup.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the major fruit trees in Egypt (El-Assar et al., 2005). Date fruit consumption is an important source of supplying mineral and vitamin elements in a balanced nutrition regime (Al-Shahib and Marshall, 2003). Research proves that when dates are eaten alone or in mixed meals with plain yoghurt they have low glycaemic indexes (Yousif et al., 1996; Miller et al., 2003). The good news is that consumption of dates may also benefit in glycaemic and lipid control of diabetic patients (Miller et al., 2002, 2003). Date fruit has anti-tumor activity (Ishurd and Kennedy, 2005), antioxidant and anti-mutagenic properties (Vayalil, 2002; Mansouri et al., 2005). The fruit has been recommended in folk remedies for the treatment of various infectious diseases and cancers (Duke, 1992). Dry date fruits are used in Indian traditional medicine after child birth as immunostimulants (Puri et al., 2000). Aqueous date extract was also found to significantly inhibit lipid peroxidation and protein oxidation in a dose-dependent manner (Allaith and Abdul Ameer, 2008). Furthermore, Al-Shahib and Marshall (2003) concluded that, in many ways, dates may be considered as an almost ideal food, providing a wide range of essential nutrients and potential health benefits.

The aim of research was using the date extract as part of an aqueous phase used in reconstituting skim milk powder, processed yoghurt and evaluate the nutritional values of the new product.

MATERIALS AND METHODS

Skim milk powder (low heat, origin USA) was reconstituted in distilled water and left overnight at 4°C to allow full hydration. Dried date fruits came from the local market. Folin-Ciocalteu reagent and 2,4,6-Tris [2- pyridyl]-s-triazine (TPTZ) were obtained from Fluka Chem. Co (Buchs, Switzerland), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and the reagents, (S)-(-)-6-Hydroxy- 2,5,7,8- tetramethylchroman -2-carboxylic acid

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(TROLOX) from (Sigma, St. Louis, Mo., USA), and gallic acid from (MP Biomedicals. Inc. (Eschwege, Germany). Sarter: freeze dried culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarious* subsp. *thermophilus* (1:1) were obtained from Chr. Hansens laboratories, Denmark.

Preparation of Date Palm Syrup (Aqueous Extract of Date Fruit/Date Juice)

Dried date fruits (500 g) were grinded with a mechanical set (to increase the surface area), infused in 1000 ml hot water then stirred for 2h and allowed to stand in a refrigerator overnight to fully extract. The raw date palm syrup was extracted by squeezing the mixture through cheese cloths. The date fruit extract was collected and used with different concentrates as a part of water (2.0, 4.0, 6.0, 8.0 and 10% v/v) in reconstituted skim milk powder that was prepared for processing yoghurt (Vayalil, 2002).

Determination of Total Soluble Solids (TSS) of Prepared Date Palm Syrup (Brix°)

Total soluble solids in the raw date palm syrup were measured using an Abbe Mark II digital refractometer (Leica Inc., Buffalo, NY) by placing 0.5 g syrup on the lens and reading the sample for temperature corrected Brix.

Yoghurt Manufacture

Skim milk powder was reconstituted in distilled water including date palm syrup to give 14% total solids. Reconstituted milk was heat-treated at 90°C for 10 min, then cooled to 43°C, starter was added at the rate of 3% and incubated at 43°C for 4h until coagulation occurred and samples reached to pH 4.3. Yoghurt was then refrigerated at 5°C for 12 d and subsequent analysis. Plain yoghurt was produced by the same procedure. Samples were duplicated.

Physical Properties of the Yoghurt

1. Sensory Evaluation. Yoghurt samples were served in plastic plates labeled with three-digit codes from a random number table. The quality properties that were evaluated were color, firmness, smoothness, taste, sweetness, sourness, flavor and overall acceptance. The sensory scores of produced types of yoghurt was done on a 9-point hedonic scale with 1=dislike extremely and 9=like extremely (Larmond, 1980).

2. Apparent Viscosity of Yoghurt (cP.s). Apparent viscosity was based on measuring resistance to a rotating spindle (Brookfield Model DV III, Programmable rheometer) Depending on time of shearing test samples were subjected to shear rate at a spindle speed of 50 rpm and spindle rotating velocities, at constant temperature (25°C) for 5 min. The instrument was equipped with an 18 measuring head. Samples were allowed to relax (more than 10 min) prior to measuring their viscosity. All apparent viscosity measurements were expressed in cintipoise seconds (cP.s), performed in duplicate.

Determination of the Antioxidant Activities in Yoghurt during Storage

The antioxidant activity in yoghurt (14% TS) was monitored during storage (0, 3, 6, 9, and 12 days).

1. Determination of Total Phenols Content. The method of Zheng and Wang (2001) was followed in determining the total phenol compounds in yoghurt using the Folin Ciocalteu Reagent (FCR) and gallic acid as a standard solution. Aliquots (20 µl) of the diluted extracts were mixed with 100 µl of the Folin-Ciocalteu phenol reagent and 300 µl of 20% Na_2CO_3. The absorbance was read with a SP-2000UV UV/V is spectrophotometer at 765 nm. The total phenol contents were calculated from a standard curve of diluted gallic acid solution and expressed as gallic acid equivalent in (GAE) mg/100 ml extract.

2. Measurement of DPPH Radical Scavenging Activity. The DPPH free radical scavenging activity of yoghurt was assessed according to the method mentioned at (Larrauri et al., 1998) with some modifications. Briefly, 40 µl of the different blends of yoghurt samples were mixed with 2.9 ml of 0.1 mM DPPH solution in methanol and the absorbance was measured at 517 nm. A standard curve was prepared for the reaction

between 40 µl of Trolox solutions (0.5 mM) and DPPH the same as the samples. The scavenging activity of the different samples was measured from the prepared standard curve and expressed as µmoles Trolox Equivalents/100 ml sample (TE).

3. Measurement of the Ferric Reducing Antioxidant Power (FRAP). The method of Benzie and Strain (1996) was followed in determining the FRAP. Aliquots of 100 µl of blended yoghurt samples were mixed with 3 ml FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm after incubation at 37°C for 10 min. FRAP values were obtained from a standard curve prepared ferrous sulphate ($FeSO_4.7H_2O$) solutions (0.1-3.0 mmol/L) and data were expressed as mg Fe^{2+}/100 ml (FRAP value). The highest antioxidant values during storage were recorded from samples, the nutritional value was evaluated as chemical composition, and some micronutrient (HCl-soluble minerals) and (folate and C vitamins) were compared to plain yoghurt.

Chemical Composition of Yoghurt

The pH, acidity, moisture, protein, fat and total solids of yoghurt containing date products were determined. The pH was measured using a Jonway 705 pH meter. Titrable acidity was determined as lactic acid by titrating with 0.1 N NaOH using phenolphthalein as an indicator. Total solids content was determined in a laboratory oven at 105°C for 24h, and total protein was assayed by the Kjeldahl method (Ling, 1963).

Liquid High-Performance Chromatographic Determination of Water-Soluble Vitamins (Vit. C and Folic Acid in Yoghurt)

A weight of 5 g of prepared yoghurt sample was stirred well with 70 ml of 0.02% EDTA in 2 N H_2SO_4. The blend was transferred quantitatively to a 100-ml volumetric flask and the volume made to mark with 0.02% EDTA solution. An aliquot of the blend was centrifuged at 7000×g for 5 rain, then analyzed for vitamin C according to the HPLC method of Ilic and Ashoor (1988).

Determination of HCI-Soluble Mineral (K, Ca, Mg, P, Fe and Z) Concentrations in Yoghurt

Minerals (K, Ca, Mg, P, Fe, and Zn) content of the yoghurt was determined by atomic absorption spectrophotometer (Varian spectra AA 220) (Adnan et al., 1999).

Statistical Analysis

Statistical analysis was performed by using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1988). The least significant difference test (LSD) was used to test differences between means ($P \leq 0.05$).

RESULTS

Physical Properties of the Yoghurt

1. Sensory Evaluation. Yoghurt having 10% date palm syrup recorded the highest sensory scores (Table 1) and had a 35.8 °Brix level (SS).

2. Apparent Viscosity of Yoghurt (cP.s). The apparent viscosity of yoghurt measured as function of shearing time showed greater reduction of apparent viscosity with time of shearing. In yoghurt made from skim milk mixed with different concentrations of aqueous extract of date fruit dry dates (2.0, 4.0, 6.0, 8.0, and 10%) apparent viscosity at initial stress was 41, 37, 34, 33, and 30.0 cP.s whereas plain yoghurt recorded 46.0 cP.s (Fig. 1). Apparent viscosity had a lower viscosity in increased date palm syrup concentration. The apparent viscosity with time of shearing, decreased. There were significant differences between concentration at time 0.0, 0.5, 1, 1.5, 2, and 2.5 min. However, at 3 min no significant differences between the concentrations were observed.

Determination of the Antioxidant Activities in Yoghurt during Storage

1. Total Phenols Content. Soluble phenolic content of yoghurt made with skim milk-

date palm syrup increased significantly (P<0.05) with increasing the concentration of date palm syrup 2, 4, 6, 8, and 10% in yoghurt produced whereas decreased by storage time (0, 3, 6, 9, and 12 days) in the fixed concentration. Plain yoghurt having 248 mg GAE/100 ml sample at zero day storage decreased to 210 mg GAE/100 ml sample at 12 days storage and yoghurt-10% date syrup had 306 mg GAE/100 ml yoghurt sample at zero day also decreased to 260 mg GAE/100 ml yoghurt sample at 12 days storage (Fig. 2).

2. DPPH Radical Scavenging Activity. Results showed that scavenging capacity of yoghurt significantly increased (P<0.05) with increasing the concentration of date palm syrup addition. Scavenging capacity of yoghurt-10% date palm syrup was 103.3 TE mg/100 ml at zero day storage and values decreased to 80.3 TE mg/100 ml at 12 days storage. Scavenging capacity of plain yoghurt was 68.9 TE mg/100 ml at zero day storage and decreased to 40.0 TE mg/100 ml at 12 days storage (Fig. 3).

3. The Ferric Reducing Antioxidant Power (FRAP). This antioxidant power significantly increased (P<0.05) with increasing the date palm syrup concentration in the yoghurt and decreased with extended storage. The antioxidant power of yoghurt having 10% date palm syrup was 43.3 mg Fe^{2+}/100 ml at zero day storage, then decreased to 33.5 mg Fe^{2+}/100 ml after 12 days storage. The antioxidant power of plain yoghurt was 35.4 mg Fe^{2+}/100 ml at zero day storage, then reduced to 23.0 mg Fe^{2+}/100 ml after 12 days storage (Fig. 4). From previous results yoghurt-10% date palm syrup recorded the highest antioxidant values after 12 days storage, so it was chosen as a new product (functional yoghurt) and its nutritional value was evaluated compared with plain yoghurt.

Evaluation of the Nutritional Values of Yoghurt-10% Date Juice Compared with Plain Yoghurt

1. Chemical Composition of the Chosen Yoghurt-Date Sample Compared to Plain Yoghurt. Means for chemical composition (pH, acidity, moisture, protein, fat and total solids) of yoghurt containing 10% date palm syrup and plain yoghurt are presented in Table 2. Addition of date palm syrup decreased the protein content, acidity and moisture while increasing the total solids of the yoghurt.

2. Determination of Some Micronutrients: Vitamins and Minerals. A rapid, simple and reliable liquid chromatographic method had been developed for the simultaneous determination of water-soluble vitamins in yoghurt. Results showed no significant difference in vitamin C concentration in both yoghurts whereas folate concentration had increased significantly (P<0.05) in yoghurt-10% date juice compared to plain yoghurt (Table 3). The HCl-soluble mineral content (potassium, calcium, phosphorus, manganese, iron and zinc) in the plain yoghurt sample were 1978, 1100, 1002, 155, 3.1 and 5.2 ppm whereas in yoghurt-10% date juice these were 3744, 1700, 1704, 360, 10.8, and 17.8 ppm, respectively, with significant differences (P<0.05) as shown in Table 4.

DISCUSSION

Participants found the sensory attributes of yoghurt flavored with date palm syrup to be very acceptable. Previous studies were obvious that date fruit extracts significantly increased or decreased gastrointestinal transit (GIT) in mice (Al-Qarawi et al., 2003), and that date fruit extract had strong antioxidant and antimutagenic properties (Vayalil, 2002). Data presented in this study demonstrated that yoghurt containing 10% date palm syrup had a significantly higher sensory evaluation, higher taste rating, higher sweetness rating compared to plain yoghurt. The higher taste and sweet values related to the date syrup that contains a high percentage of carbohydrate. Although increasing the extract of date fruit in yoghurt caused leasing in the apparent viscosity, the yoghurt with 10% date syrup gave acceptable consistency, it was specially processed without stabilizer. The high levels of sugar bind moisture effectively thus preserving the fruit by preventing bacterial growth (Al-Shahib and Marshall, 2003).

Yoghurt enriched with 10% date palm syrup had the highest total phenolics content, hydrogen-donating capacity and ferric reducing antioxidant power compared to the other sample with all storage times. The antioxidant activity of date fruits have also

been assessed and reported by other researchers using different methods. The predominant phenolics found in date fruits are very active as antioxidants and the antiradical activity in dates was highly correlated to the phenolic contents (Mansouri et al., 2005). The antioxidant activity of the yoghurt-10% date palm syrup was attributed to the presence of phenolic compounds (Ishurd and Kennedy, 2005; Allaith and Abdul Ameer, 2008).

Vitamins and minerals are classified as micronutrients which the body daily requires. This concentrate of date palm syrup (10%) in yoghurt was compared with plain yoghurt in both some major minerals and vitamins as a required nutritional quality of the product that provides a wide range of potential health benefits. The HCl-soluble mineral content of the yoghurt-10% date juice was higher than the plain yoghurts. The new product was also rich in calcium that helps strengthening bones and is particularly beneficial to young children to prevent rickets, and brittle and weak bones in adults (Mackie et al., 1989). Adequate intake of calcium and other nutrients from dairy has also been demonstrated to help reduce the risk of high blood pressure (Miller et al., 2000). This yoghurt was also rich with potassium which regulates the water balance in the body and provides the appropriate alkaloidal features for body fluids, in addition to stimulating the kidneys to expel toxic bodily wastes (Lindinger, 1995). Potassium is not stored in the body, and much is lost in perspiration, it must be continually replenished. Yoghurt with date palm syrup has a large amount of iron which controls the synthesis of hemoglobin in the red blood cells and ensures an appropriate level of red cells in the blood (MacPhail, 2007). Zinc in new yoghurt was also more than in plain yoghurt. Zinc is believed to play a valuable role in the healing process, blood stability, and mental functions and in keeping a proper alkaline balance in the body (Hamrick and Counts, 2008).

Yoghurt enriched with 10% date palm syrup was exceedingly rich in folic acid. Folic acid, a B9 vitamin is of great importance to pregnant women. The need for folic acid thus rises significantly during pregnancy and the daily requirement doubles (Goh and Koren, 2008). Folic acid plays a particularly important role in cell division and in the formation of the genetic structure of the cell (Calvet and Chadwick, 1994). It has become clear that folate plays important roles not only in the prevention of neural tube defects (Czeizel and Dudás, 1992), but possibly also in the etiology of cardiovascular diseases (Verhoef et al., 1996) and cancer (Giovannucci et al., 1993; Giovannucci et al., 1998). Folate helps in the metabolism of several amino acids (Shimakawa et al., 1997).

CONCLUSION

Date palm syrup provides unique functionality when used with milk in processing yoghurt including sweetening, flavoring and increasing nutritional quality. Yoghurt enriched with 10% date palm syrup has a smooth texture, is mildly sour and has a pleasant flavor besides the abundance of nutritional values that provides lots of health benefits. The main benefit of the new product yoghurt is that it provides more content of HCl-soluble mineral that we need to stay healthy. Phosphorus works with calcium to help with bone strength and growth, potassium helps to keep your muscles working correctly and zinc is important for cell growth and repair. Yoghurt enriched with 10% date palm syrup is also a good source of folate which increases the chances of achieving nutritional recommendation. Numerous health benefits beyond its nutritional value have been associated with consuming yoghurt enriched with 10% date palm syrup.

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Tables

Table 1. Average scores of sensory evaluation of yoghurt enriched with different concentrations (0-10%) date palm syrup.

Yoghurt	Color	Firmness	Smoothness	Taste	Sweetness	Sourness	Flavor	Acceptability
Plain yoghurt	8.2	7.5	8.6	7.6	5.5	7.8	6.5	6.6
Yoghurt-2%	8.1	7.4	8.5	7.6	5.6	6.7	6.9	6.7
Yoghurt-4%	8.0	7.4	8.4	7.9	5.6	6.6	7.0	7.5
Yoghurt-6%	7.8	7.2	8.2	8.1	5.8	6.4	7.2	7.7
Yoghurt-8%	7.6	7.0	7.8	8.3	6.2	6.4	7.7	7.9
Yoghurt-10%	7.4	6.7	7.5	8.5	7.6	6.2	7.8	8.2

[1] 9-point hedonic scale was used with (1) = dislike extremely and (9) = like extremely.
[2] Means within a column not followed by a common letter are different ($P \leq 0.05$).

Table 2. Chemical composition of yoghurt containing 10% date juice and plain yoghurt.

Yoghurt	pH	Moisture %	Protein %	Total solids %	Acidity %
Plain yoghurt	4.5	85.91	3.55	14.1	0.98
Yoghurt-10% date juice	4.5	85.43	3.45	14.6	0.94

All data are insignificant ($P>0.05$).

Table 3. Vitamins contents of yoghurt.

Yoghurt	Vitamin C (mg)	Folic acid (mcg)
Plain yoghurt	120.1^{a}	1.19^{b}
Yoghurt-10% date juice	120.2^{a}	4.38^{a}

- Folate (folic acid or folacin) is a water-soluble B9 vitamin
-Dissimilar superscripts at the same column are significant ($P<0.05$).

Table 4. HCl-soluble mineral content (K, Ca, P, Mg, Fe and Zn) (ppm) in yoghurt.

Yoghurt	K	Ca	P	Mg	Fe	Zn
Plain yoghurt	1978^{b}	1100^{b}	1002^{b}	155^{b}	3.1^{b}	5.2^{b}
Yoghurt-10% date juice	3744^{a}	1700^{a}	1704^{a}	360^{a}	10.8^{a}	17.8^{a}

Dissimilar superscripts at the same column are significant ($P<0.05$).

Figures

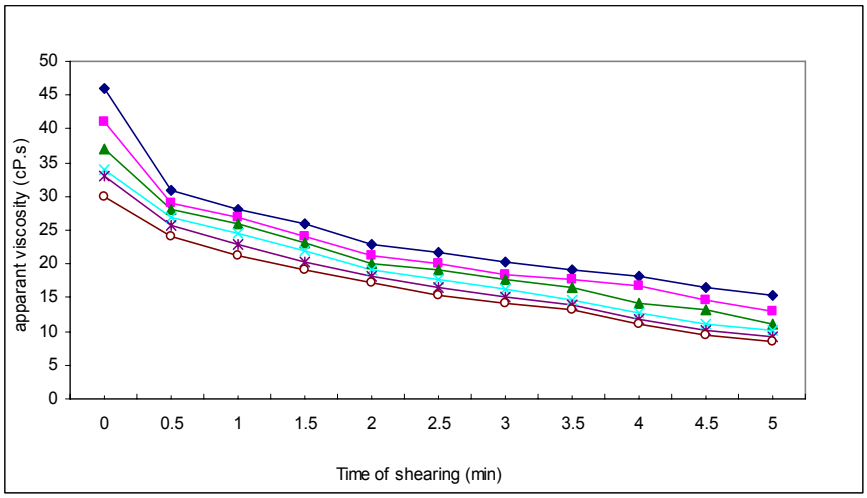

Fig.1. Shows the dependency of the apparent viscosity of yoghurt (skim milk mixed with different concentration of dry dates 14% TS pH 4.5) with the time at a constant shearing for 25 °C. [(-♦-) without add; (-▄-) add + 2%;(-▲-) add 4%; (-x-) add 6%; (-*-) add 8% (-o-) add 10%] n = 3. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05).

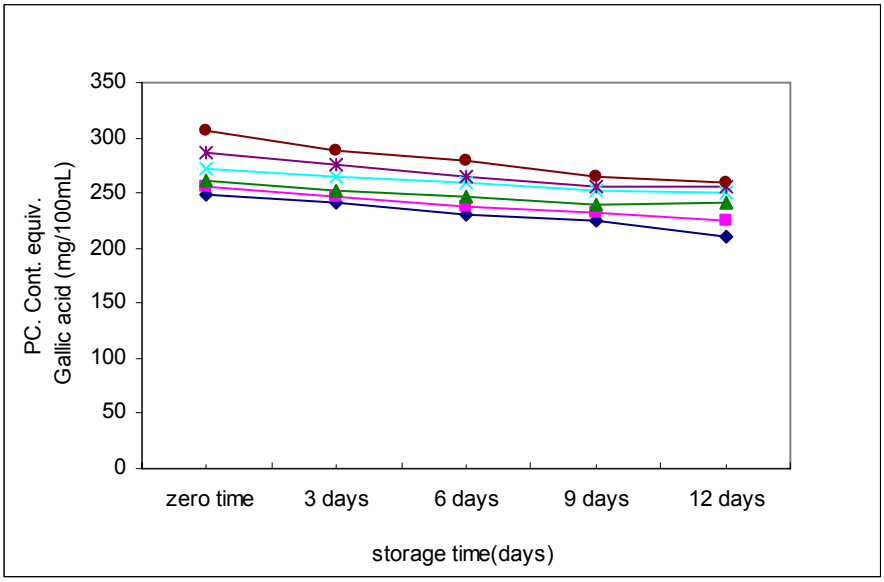

Fig. 2. Effect of storage time on phenolic compound content in milk with different conc. of dry dates additives [(-♦-) without add; (-▄-) add 2%;(-▲-) add 4%; (-x-) add 6%; (-*-) add 8% (-o-) add 10%.]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05).

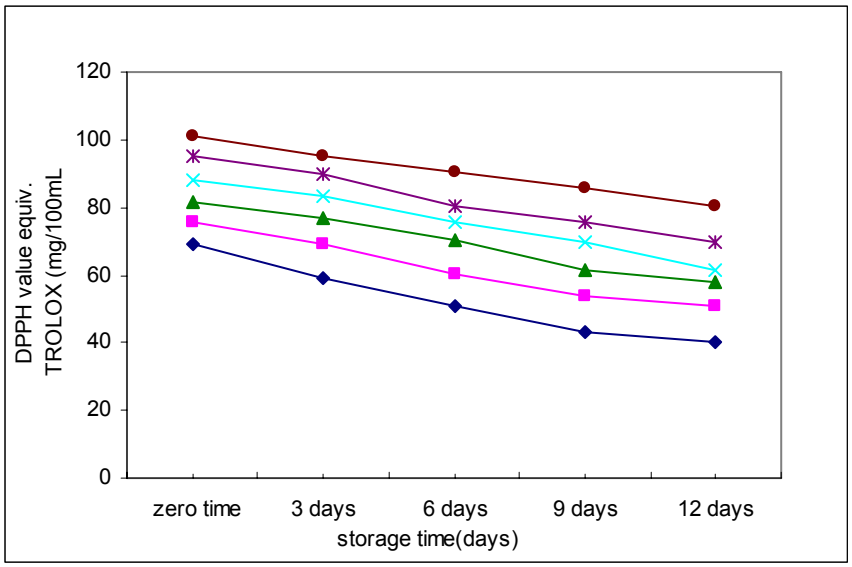

Fig. 3. Effect of storage time on antioxidant activity of using DPPH method in milk with different conc. of dry dates additives [(-♦-) without add; (-■-) add 2%;(-▲-) add 4%; (-x-) add 6%; (-*-) add 8% (-o-) add 10%.]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05).

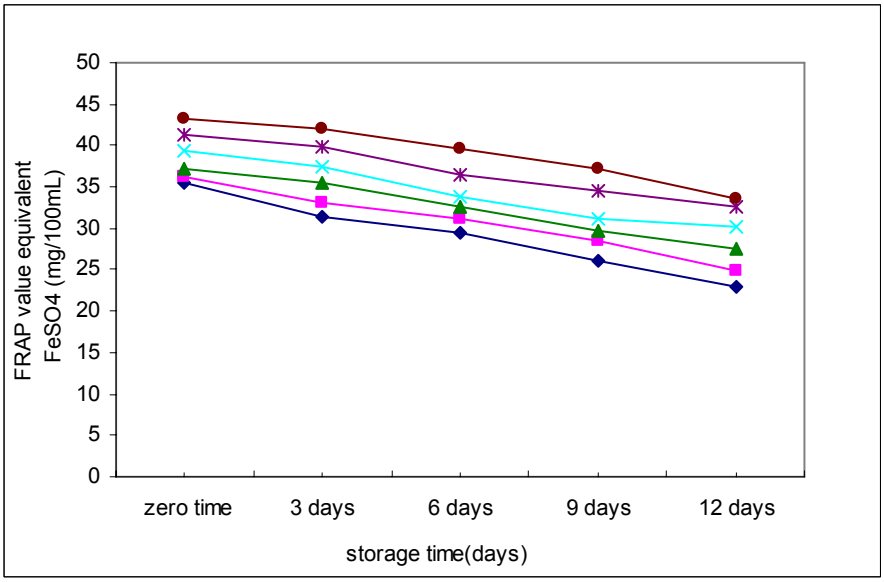

Fig. 4. Effect of storage time on antioxidant power (FRAP value) using FRAP method of milk with different conc. of dry dates additives [(-♦-) without add; (-■-) add 2%;(-▲-) add 4%; (-x-) add 6%; (-*-) add 8% (-o-) add 10%.]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05).

Processing Dates of Low Market Value into Flour: Evaluation of Quality and Storage Stability

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Keywords: Morocco, *Phoenix dactylifera* L., variety, date, processing, flour, quality

Abstract

Flour production is a way of valorizing dates, especially those of low market value. This flour can be used as an ingredient in biscuits, cakes, infant foods, etc. This work aims to assess the suitability of Moroccan dates for processing into flour and determine the nutritional, organoleptic and commercial qualities of the date flours. Six varieties of dates were studied: 'Ademou', 'Bouijjou', 'Bouskri', 'Bouslikhene', 'Jihel' and 'Oum-N'hal'. The flour quality was evaluated by studying, among other criteria, water absorption index (WAI), water activity (a_w) and sorption curves, and by carrying out sensory analysis. The storage stability was evaluated by keeping the flour at 25 and 45°C for 3 and 6 months. This work has highlighted the high suitability of 'Bouskri' and 'Bouijjou' for processing into flour. 'Bouslikhene' was not selected because of the sticky and doughy texture of its dried pulp. Beside high processing yields reaching 75.3%, the date flours showed interesting characteristics in terms of nutritional and organoleptic qualities. The WAI provided information on distinct rheological characteristics of the different flours and a_w data, lower than 0.41, lead to good microbial and enzymatic stability. The sorption curves of date flours also showed different hygroscopic behaviors. For storage stability, time and temperature had a significant effect on the physico-chemical properties of the flour. The negative effect was mainly represented by the color browning and the loss of the floury texture especially at the temperature of 45°C. The sensory evaluation of date flour was very encouraging and showed that this flour is a promising product to enrich and/or flavor food preparations.

INTRODUCTION

The flour production is among the possible ways of adding value to the dates especially those of low commercial value. It includes the category of the by-product diversification as is the case of date paste and jam. Therefore, this technological operation is not a total date processing (Estanove, 1987).

The preparation of date flour requires hard and breakable fruits, or those which naturally dehydrate or become hard after drying. The date flour can be used as an ingredient to be incorporated in food preparations. Rich in sugars, the date flour is used in cookies, breakfasts, baby foods, etc. (Munier, 1973).

We can also evoke the traditional dates processing into flour called "Lhrissa" which is performed by the women in the date palm oasis by using the dry dates added to "Zemmita" (the steamed, roasted and ground barley flour). This local knowledge is relatively important, not only because it increases dates commercial value but also because aromatic and medicinal herbs added to "Lhrissa" (cumin, aniseed, round mint, capillary, pennyroyal, etc.) make it a very popular food for the indigenous people particularly for its nutritional and organoleptic qualities and its therapeutic virtues. "Lhrissa" is also among the main food taken by the oasis people to the pilgrimage (Harrak and Chetto, 2001).

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The aim of this research was to study the suitability of Moroccan dates for processing into flour and to evaluate the nutritional, organoleptic and commercial qualities of the date flours. It is justified by the value to be added to the national date production, the diversity of date by-products to be ensured in the market and the demand of the profession to be satisfied in the Moroccan oases.

MATERIALS AND METHODS

Choice of Date Varieties

We studied six date varieties: 'Ademou', 'Bouijjou', 'Bouskri', 'Bouslikhene', 'Jihel' and 'Oum-N'hal'. They were chosen for semi-soft and dry consistency, fibrous nature or low commercial quality. The dates were harvested at the last stage of ripening (tamar stage) in 2007 at the "Nebch" Experimental Domain, INRA, Zagora.

Evaluation of the Date Suitability for Processing into Flour

1. Pulp Content. The pulp content, expressed as date pulp weight on date weight, was determined for each variety by using a sample of 500 g.
2. Fibrous Nature. The fibrous nature of the rag (the fibrous part of the pulp surrounding the stone) was observed using a sample of 10 dates randomly taken for each variety.
3. Humidity. The dates humidity is determined according to AOAC method No. 920.151 (AOAC, 1990) by drying the ground pulp dates in a vacuum oven at a temperature of 70°C during 48 hours. The humidity is expressed as g of water per 100 g of fresh matter.
4. Brix. The Brix or the soluble solids, expressed as °Bx, was determined at 20°C by refractometry according to AOAC methods No. 932.12 and No. 932.14C (AOAC, 1990).
5. Quality Index. The quality index "r" was calculated by dividing total sugar content (g/100 g of dry matter) by water content (g/100 g of dry matter) (Reynes et al., 1994).
6. Water Activity. The water activity (a_w) was measured at 25°C using an a_w-meter. The equilibrium between date pulp and surrounding atmosphere was reached after 3 hours.
7. Flour Yield. The flour yield was determined by dividing flour weight by date pulp weight (g of flour/100 g of date pulp).

Determination of Quality Criteria of Date Flour

1. Color. The flour color was determined by measuring the L*a*b* parameters of the Hunter Lab scale with a spectrocolorimeter for liquid. The liquid solution of flour was obtained after centrifugation during 10 min at the speed of 2800 rpm followed by filtration of a mixture prepared with a "flour: distilled water" ratio of 1:10.
2. Water Absorption Index. The water absorption index (WAI) was determined by preparing saturated and homogeneous flour dough by adding distilled water to flour. The WAI is expressed as ml of water/g of flour.
3. Humidity. The flour humidity was determined by the same method above used for determining the date humidity. It is expressed as g of water/100 g of flour.
4. Brix. The flour Brix was determined by the same method above used for determining the date Brix.
5. pH. The pH was determined according to AOAC method No. 981.12 with a pH meter (AOAC, 1990) by using flour dough prepared with a "flour: distilled water" ratio of 1:1.
6. Total Titratable Acidity. The total titratable acidity was determined by titration at pH 8.1 according to AOAC methods No 920.149(c), No 942.15A and No 942.15B (AOAC, 1990) by using flour dough prepared with a "flour: distilled water" ratio of 1:1. The acidity is expressed as g of citric acid/100 g of flour.
7. Cellulose. The determination of cellulose was carried out according to the Voiret method (Lecoq, 1965) by halogenations and successive alkaline washing to dissolve the various components of encrusting cellulose until obtaining white and pure cellulose. The cellulose is expressed as g/100 g of dry matter.
8. Ash. The ash was determined by incineration at 500°C of date flour in an electric furnace according to AOAC method No 940.26 (AOAC, 1990). The ash is expressed as

g/100 g of dry matter.
9. Water Activity. The water activity was determined by the same method above used for determining the date a_w.
10. Sorption Curves. The sorption curves of flours were obtained by the saturated salts method at 25°C according to the Conway cells method (Labuza et al., 1976; Jannot, 2003). The flour water content was determined, after equilibrium, for each sample kept at various a_w by drying in a vacuum oven at 70°C during 48h. Thus, the determination of the water contents and their corresponding a_w provided spots of the sorption curves.
11. Sensory Analysis. The sensory analysis of date flours was achieved by a tasting panel of 10 persons by using a hedonic scale of ranged scores from 1 to 9 for respectively less accepted and more accepted flour criterion. The criteria evaluated are color, taste, odor, texture and overall appearance.

Evaluation of Storage Stability of the Date Flour

The date flours were stored in hermetic plastic boxes at two temperatures (25 and 45°C). The stability evaluation was carried out after 3 and 6 months of storage. The evaluated criteria included humidity, a_w, Brix, pH, total acidity, color (L*a*b*) and ash. The color and the texture of the flours were also evaluated by visual observations.

Data Statistic Analysis

The analysis of variance (ANOVA) with one classification criterion was used to compare the date varieties and their flours before and after storage. The Student-Newman-Keuls method allowed, after having rejected the hypothesis of equality of means, to seek, criterion by criterion, the homogeneous groups of means. The hierarchical classification was also carried out for comparing the data of the sensorial analysis of date flours. The calculations were performed by the statistical software Minitab.

RESULTS AND DISCUSSION

Suitability of Date Varieties for Processing into Flour
1. Pulp Content. For the best yield of flours, the required dates are those rich in pulp. The pulp content is higher for 'Bouslikhene' (87.99%), followed by 'Jihel' (85.23%), 'Bouskri' (85.07%), 'Bouijjou' (83.77%) and 'Ademou' (81.69%). The 'Oum N'hal' variety has the lowest pulp content (77.47%).
2. Fibrous Nature. Depending on the fibrous nature of the rag, the 6 varieties were rich in fibers. 'Ademou', 'Bouijjou' and 'Bouskri' were more fibrous than 'Bouslikhene', 'Jihel' and 'Oum-N'hal'. The fibrous nature of date pulp is an interesting technological property for the flour production in particular of dry dates or those which easily dehydrate after harvest.
3. Humidity. 'Bouskri' was the more humid (16.39%), followed by 'Oum-N'hal' (14.78%) and 'Jihel' (8.89%) while the driest varieties were 'Bouslikhene' (6.38%), 'Ademou' (6.74%) and 'Bouijjou' (6.82%). The humidity difference between the 6 varieties was very highly significant according to the ANOVA analysis. An additional drying of these dates could facilitate grinding for flour production. Lecoq (1965) gave the example of the Algerian dry variety, 'Degla-Beida', having around 15% of humidity and nutty flavor which is suitable for flour production.
4. Quality Index. The difference between the data of the quality index, or hardness index "r", of the 6 varieties was very highly significant according to the ANOVA analysis. They ranged from 4.8 to 13.0 and ranked the 6 varieties in the category of dry consistency: 'Bouijjou' (13.0), 'Bouslikhene' (12.9), 'Ademou' (11.7), 'Jihel' (9.1), 'Oum-N'hal' (5.1) and 'Bouskri' (4.8). In fact, the "r" data largely exceeded the value of 3.5 above which dates are considered of dry consistency ($r>3.5$).

According to Harrak et al. (2005), the quality indexes were 2.8, 3.0 and 3.3 respectively for 'Bouskri', 'Oum-N'hal' and 'Jihel', consequently ranked in the category of semi-soft consistency ($2<r<3.5$). The 'Bouijjou' and 'Bouslikhene' varieties were

ranked in the category of dry consistency ("r" were 4.8 and 6.3 respectively). The higher indexes found in this work could be due to the dates over drying before or after harvest. Indeed, it is known that the 'Bouskri' and 'Oum-N'hal' dates dry after harvest. The relatively hard consistency of these dates could facilitate grinding for flour production.

5. Flour Yield. Except 'Bouslikhene', the five other varieties have produced flour. The flour yields ranged from 63.2% for 'Oum-N'hal' to 75.3% for 'Bouskri' and 'Jihel'. The 'Ademou' and 'Bouijjou' varieties have recorded respectively 72.5% and 74.0% for yields. It should be noticed that the more important the pulp content is, the higher the flour yield.

Drying durations were not closely related to the initial water content of dates. These durations rather depended on the ability of dates to release water during drying. For example, the 'Bouskri' dates dried up more easily compared to the other varieties. Moreover, this variety is characterized by a rapid drying after harvest. The 'Bouslikhene' dates showed a great resistance to release water during drying. Thus, the rank of varieties, according to the increasing order of drying durations, was as follows: 'Bouskri' < 'Jihel' < 'Ademou' < 'Bouijjou' < 'Oum-N'hal'.

According to flour yield and drying duration, 'Bouskri' and 'Jihel' could be characterized by a good grinding quality: they gave good flour yield with less energy.

This rank has been preserved for 'Bouskri', 'Ademou' and 'Oum-N'hal' according to the decreasing order of the floury texture of their grinded dried pulp. The varieties which had a very fibrous rag presented a good floury texture of their flours (Fig. 1): 'Bouskri' > 'Bouijjou' > 'Ademou' > 'Jihel' > 'Oum-N'hal'.

The 'Bouskri', 'Jihel' and 'Oum-N'hal' varieties are among the dates which are processed into the traditional flour "Lhrissa" prepared by the women of the Drâa oasis. This flour is qualified excellent for 'Bouskri', very good for 'Jihel' and medium for 'Oum-N'hal'. The latter is especially used after its natural postharvest drying. 'Bouskri' is also appreciated for its delicious taste (Zirari et al., 2003).

Concerning the date pulp of 'Bouslikhene', it preserved its doughy and sticky texture even after a relatively long drying time (Fig. 2). Consequently, we could deduce that a high hardness index cannot ensure good date suitability for processing into flour.

Quality Criteria of Date Flours

The ANOVA of date flours criteria (humidity, a_w, Brix, pH and acidity) has revealed very highly significant differences between the studied varieties. For the WAI, the difference was highly significant. On the other hand, no significant difference was recorded for the ash and the cellulose content.

1. Water Absorption Index. The WAI recorded for date flours (Table 1) showed that 'Bouskri' absorbed less water (0.56 ml/g) and 'Ademou' absorbed more water (1.25 ml/g) compared to the other varieties. The differences recorded for WAI informed about the distinct rheological characteristics of flours if they are used in liquid or semi-solid food preparations. In addition, the higher the WAI, the higher the saturation effect of the flour consumption. This is the case of cactus cladodes flour which absorbed a quantity of water largely higher (5.6 ml/g) than date flour (Saenz, 2006).

2. Humidity. The humidity of the date pulp highly decreased after drying. The humidity loss varied from 3.80% for 'Ademou' to 13.34% for 'Oum-N'hal'. 'Bouskri' flour recorded the highest humidity (3.49%) while 'Oum-N'hal' flour had the lowest humidity (1.44%). The 'Bouslikhene' dried pulp contained only 1.71% of humidity without providing flour (Fig. 3).

3. Brix. The date Brix which differed very high significantly increased largely after drying the 5 varieties having shown a good suitability for processing into flour (from 4.0°Bx for 'Bouijjou' to 17.4°Bx for 'Oum-N'hal'). The highest and the lowest Brix were recorded respectively for 'Jihel' flour (93.6°Bx) and 'Bouskri' flour (92.1°Bx). The Brix of 'Bouslikhene' dried pulp increased by 13.5°Bx and recorded 95.5°Bx (Fig. 4).

4. pH and Total Titratable Acidity. The pH and the total titratable acidity of the date flours varied respectively from 4.95 to 6.30 and from 0.410 to 0.990 g of citric acid/100 g.

The flours with the highest acidities and the lowest pH are those of 'Bouijjou' and 'Ademou'. On the other hand, the less acid flour (tending toward neutrality) is that of 'Bouskri' with a pH of 6.30 and an acidity of 0.410 g of citric acid/100 g (Fig. 5).

5. Cellulose. The 'Ademou' variety was the richer in cellulose, followed by 'Oum-N'hal' and 'Bouskri'. The lowest cellulose contents were recorded for 'Bouijjou' and 'Bouslikhene' flours (Table 1). These results are partially in concordance with the visual observations concerning the fibrous nature of the rag and the floury texture of the grinded dried pulp.

6. Ash. 'Bouskri' flour was characterized by the highest ash content (2.20 g/100 g of dry matter), followed by the 'Bouijjou' and 'Ademou' flours (Table 1). These mineral matter contents provided information on a good nutritional value of date flours.

7. Water Activity. The date a_w which differed very high significantly between the 6 varieties, decreased after dates drying (from 0.07 for 'Bouijjou' to 0.28 for 'Oum-N'hal'). The date flours presented a_w ranging from 0.41 for 'Bouskri' to 0.27 for 'Oum-N'hal' (Fig. 6). Such a_w data recorded in the date flours can ensure a good microbial and enzymatic stability. Although, these a_w could slowly provoke the Maillard reactions at room temperatures. For this purpose, the attention should be attached to these reactions, which would cease only below 0.25, for the stored date flours because of the undesirable colors and flavors that they can generate.

8. Sorption Behavior. The sorption behavior of date flours was determined by the establishment of the sorption curves. These curves predicted in what way and to what extent the exchange of water will be done between the flours and the surrounding atmosphere. Indeed, they revealed the flour hygroscopicity character, i.e., its ability to hydrate depending on the surrounding humidity (Delmer, 1976). In the date flours sorption isotherms (Fig. 7), we have observed three zones that are generally distinguished on the sorption isotherms. Zone 1 corresponds to high bonding energies of the water.

In zone 2, the water bondings gradually relax while their mobility increases. The slope of the curve is such that to a low water content gradient a rapid rise of the a_w corresponds. In this zone, we have clearly noted the differences in the sorption behavior between date flours. Indeed, the 'Oum-N'hal' and 'Bouijjou' flour' curves showed that these flours are somewhat hygroscopic, resistant to the water absorption offered by the surrounding atmosphere. Conversely, the 'Bouslikhene' dried pulp curve was typical of a substantially hygroscopic product. The 'Jihel', 'Bouskri' and 'Ademou' flour' curves showed an intermediate behavior. In this zone too, the flour texture knows a caking (spontaneous agglomeration of the particles) (Delmer, 1976).

In zone 3, the curves bent to make accentuated slopes. The water molecules are lodged between the chains of macromolecular components and between the molecules of the soluble components. The flours' texture knows liquefaction (Delmer, 1976). In contrast to zone 2, small differences of a_w correspond to a strong differences of humidity. In this zone, the behaviors of the six varieties' products were clearly very similar.

The caking and the liquefaction are the major undesirable accidents of the texture which could occur during the storage periods especially for the hygroscopic flours' dates.

9. Sensory Analysis of Flours. The hierarchical classification using the mean scores of the sensory analysis panel showed three classes of date varieties from the more to the less appreciated: ['Jihel'], ['Oum-N'hal'] and ['Ademou', 'Bouijjou'] (Fig. 8). The appreciation of date flours of these 4 varieties by the sensorial analysis panel was very encouraging for considering date flours as promising product for its introduction, for enrichment and/or flavoring, in food preparations such as yoghurts, milk drinks and cookies.

Evaluation of the Date Flours Storage Stability

The visual observations of date flours during the storage have shown that the storage for 3 months at 25°C did not cause a noticeable change of the color. Concerning the consistency, the 'Ademou' flour has retained its floury texture. The 'Jihel' and 'Bouijjou' flours have slightly lost their floury texture but have taken again their floury

texture after shaking. Whereas, 'Oum-N'hal' flour has presented a sticky texture. During the same period of storage at 45°C, the color change of 'Ademou' and 'Jihel' flours was also unnoticeable. A slight change in the color of the 'Bouijjou' flour was observed while the color of the 'Oum-N'hal' flour varied from light to dark brown. The 'Ademou' flour has retained its floury texture. The 'Jihel' and 'Bouijjou' flours have acquired hard texture but have taken again their floury texture after shaking. The consistency of the 'Oum-N'hal' flour became less floury than the others varieties flours.

Extending the storage duration of date flour up to 6 months at 25°C generated a slight change of color. The 'Ademou' flour consistency remained less sticky than the other varieties. The 'Jihel' and 'Bouijjou' flours presented a sticky and not floury texture and the 'Oum-N'hal' flour became very sticky and acquired the cake texture. The storage at 45°C has slightly affected the color of 'Ademou'. The light brown color of 'Oum-N'hal' and 'Bouijjou' flours became dark. The white to light beige color of 'Jihel' flour became more intense. The floury texture of 'Ademou', 'Jihel' and 'Bouijjou' flours has been converted into hard and breakable texture and it has taken again after shaking. The 'Oum-N'hal' flour has acquired a very hard texture compared to the other varieties' flours.

Moreover, the evaluation of the storage effect by the ANOVA revealed for the majority of date flours criteria very highly significant differences between varieties, durations, temperatures and their interactions. The non significant effect of the temperature was especially noticed for ash and pH. But, we could consider that the increasing of the storage temperature has decreased humidity and a_w and increased acidity. The a* parameter presented very highly significant differences for the 3 factors and their interactions. This parameter, which represents the red (positive value) to green (negative value) scale, has increased when the storage temperature increased. The L* parameter which indicate the brightness degree (ranging from 0 for black to 100 for white) recorded a high value (between 88.7 for 'Ademou' and 93.8 for 'Bouijjou'). This parameter has decreased when the storage temperature increased (Table 2). Thus, the storage temperature of 45°C resulted in flours browning. Figure 9 shows the strong color change of 'Oum-N'hal' flour after 6 months of storage at 45°C.

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Tables

Table 1. Means of some date flours criteria(*).

Variety	Water absorption index (ml/g)	Cellulose (g/100 g of dry matter)	Ash (g/100 g of dry matter)
Ademou	1.25 c	2.83	1.92
Bouijjou	1.09 c	2.27	2.06
Bouskri	0.56 a	2.52	2.20
Bouslilkene	0.86 b	2.25	1.77
Jihel	0.92 b	2.41	1.82
Oum-N'hal	0.79 b	2.58	1.76

* The means followed by the same letter do not differ significantly according to the Student-Newman-Keuls method (α=0.05).

Table 2. Date flours color determined after storage.

Variété	Duration														
	0 month			3 months						6 months					
	Temperature														
	-			25°C			45°C			25°C			45°C		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Ademou	88.7	-2.0	16.7	92.7	-2.5	12.4	85.0	-3.3	44.6	92.7	-2.5	12.4	78.2	3.9	70.0
Bouijjou	93.8	-2.9	11.9	93.7	-2.7	11.8	87.3	-4.5	41.1	92.0	-2.9	15.2	77.1	4.2	68.1
Jihel	93.2	-2.3	9.8	94.4	-2.4	9.3	90.3	-5.1	26.9	93.1	-2.7	12.3	83.1	-2.3	53.7
Oum-N'hal	92.4	-3.4	16.6	86.2	-2.3	19.0	86.5	-3.7	43.6	90.1	-3.2	19.5	77.8	4.0	69.4

Figures

Fig. 1. Date flours of the 'Bouskri' and 'Bouijjou' varieties of high suitability for processing.

Fig. 2. Sticky and doughty texture of dried pulp of the 'Bouslikhene' variety.

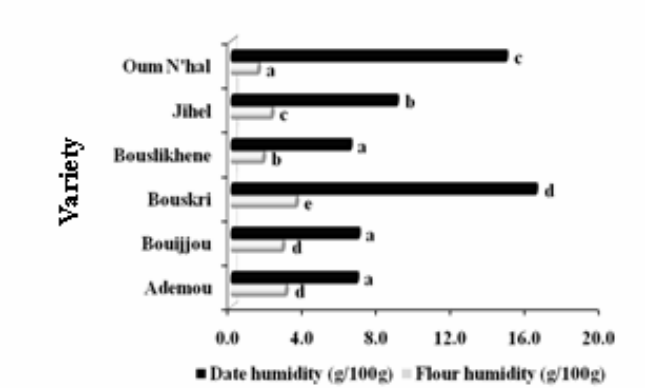

Fig. 3. Date and flour humidity of six varieties (Group means according to the Student-Newman-Keuls method (α=0.05)).

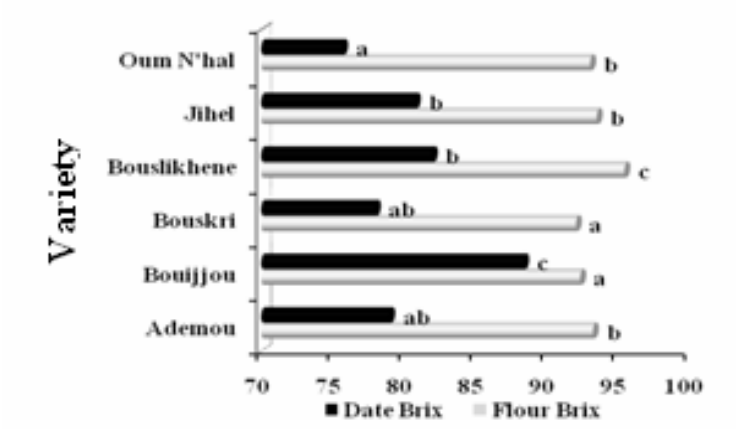

Fig. 4. Date and flour Brix of six varieties (Group means according to the Student-Newman-Keuls method (α=0.05)).

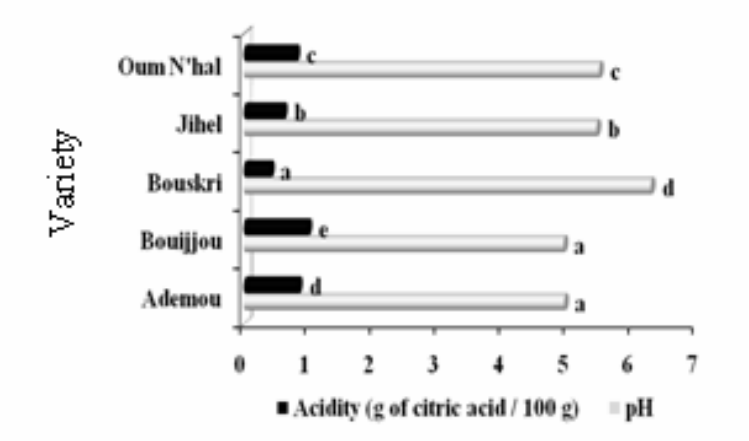

Fig. 5. pH and total acidity of date flours (Group means according to the Student-Newman-Keuls method (α=0.05)).

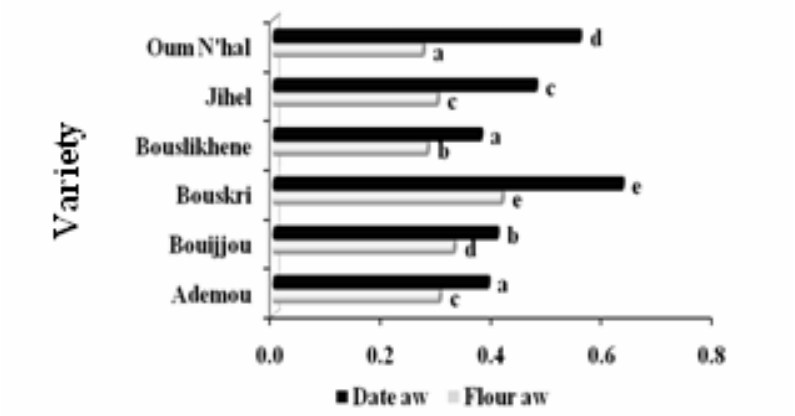

Fig. 6. Date and flour water activity (a_w) of six varieties (Group means according to the Student-Newman-Keuls method (α=0.05)).

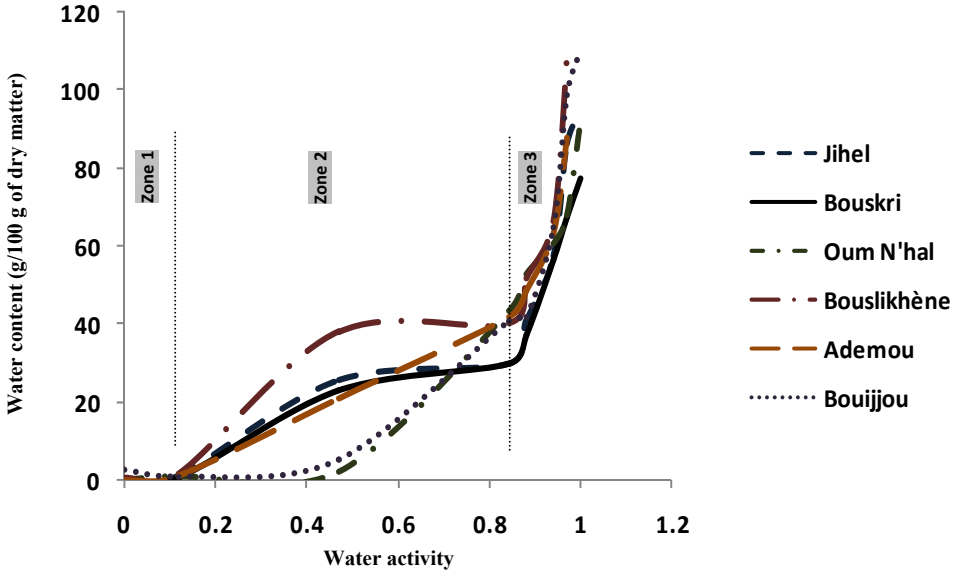

Fig. 7. Sorption isotherms of date flours.

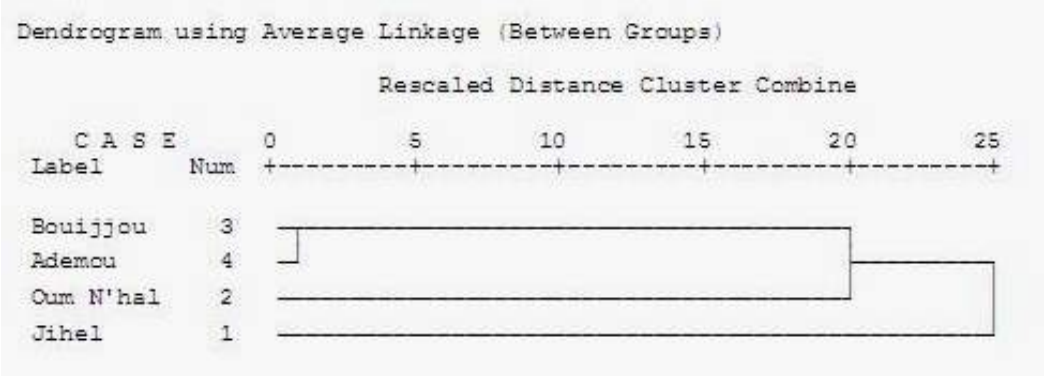

Fig. 8. Flours classification dendrogram of four date varieties based on the sensorial analysis.

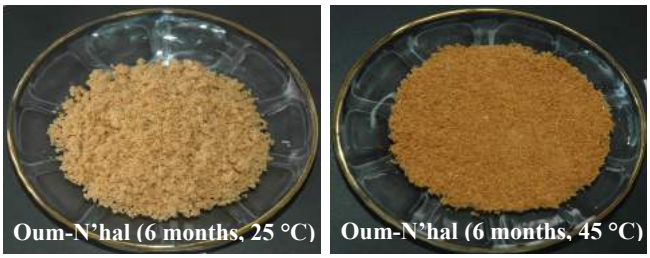

Fig. 9. The 'Oum-N'hal' variety flour after 6 months of storage: strong change in the color at the temperature of 45°C.

The Effects of Feeding Date Palm Byproducts on Meat Quality Characteristics of Sheep

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Keywords: longissimus dorsi, sarcomere length, prosopis pods, date palm fronds, concentrate

Abstract

Meat quality from 32 Omani sheep was evaluated to determine the effects of feeding four experimental diets made from a combination of two roughages and two concentrates. The roughages were urea treated palm frond silage (UTPF) and Rhodes grass hay (RGH), while the concentrates were a commercial concentrate (CC) and a local concentrate (LC). The local concentrate contained 25% ground date-palm fronds, 25% wheat bran, 20% ground prosopis pods, 15% barley and 12% dried sardines plus premix of vitamin and mineral. Four groups of animals were used with eight replicates per group: Group one: 400 gram CC plus ad libitum RGH, Group two: 400 gram LC plus ad libitum UTPE, group three: 400 grams CC plus ad libitum UTPF and Group four: 400 grams BP plus ad libitum UTPF. At the end of the feeding period (120 days), all animals were slaughtered. After 24h at 1-3°C, the M. longissimus dorsi between the 10-13 rib was removed from the left side of each carcass for meat quality evaluation. Meat quality-related measurements included ultimate muscle pH, WB-shear force value, sarcomere length, expressed juice and color (CIE L*, a*, b*) were determined. Meat ultimate pH values were not significantly different between the four diets groups ranging between 5.77 to 5.90. Lightness (L*), redness (a*) and yellowness (b*) values of longissimus dorsi muscles were comparable between the four groups. Water holding capacity measured as expressed juice was similar between the four diet groups. Sarcomere length and shear force values were also comparable between the four diet groups. This study indicated that replacing the commercial concentrate and Rhodes grass hay with a more fibrous feed made from local by-products did not produce significant affects on meat quality characteristics.

INTRODUCTION

In the Sultanate of Oman, the protein and energy requirements of growing livestock are obtained mainly from soybean and maize, the major ingredients in concentrate feeds. These ingredients are imported at high cost. The country does not produce enough animal feed due to an acute shortage in fresh water and limited utilization of arable land. However, there are some readily available livestock feeds in the natural range grazing and browsing land as well as agricultural by-products, especially those from date palms and prosopis pods (*Prosopis juliflora*). The byproducts offer a cheap potential animal feed resource in Oman, which if effectively utilized could improve the supply of animal feeds and increase profit for local farmers.

Meat quality has recently become an important aspect in the marketing of meat products in Oman. Therefore, it is time to produce high quality meat. This is also applicable for the whole Gulf region due to similarity in breeds and environmental conditions and consumer's habits. An efficient marketing system for the Omani meat industry needs more information on meat quality in relation to consumers. This study aimed to investigate the effects of replacing commercial concentrate with a concentrate made mainly from native feed resources and a roughage made from palm frond silage on

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meat quality characteristics of longissimus dorsi (LD) muscle of Omani sheep.

MATERIALS AND METHODS

Thirty-two, 6-month-old Omani male sheep were used in a feeding trial for 120 days. Animals were randomly divided into four groups of eight animals each and allocated randomly to one of four experimental diets groups. Animals for the first group were fed ad libitum chopped Rhodes grass hay plus 400 g of the commercial concentrate. Animals of the second group were fed ad libitum palm frond silage plus 400 g of the commercial concentrate. Animals of the third group received 400 g of the local by-products concentrate plus ad libitum chopped Rhodes grass hay. Animal of the fourth group received 400 g of the local by products concentrate plus ad libitum palm frond silage.

The by-product concentrate was made from 25% ground date fronds, 25% wheat bran, 20% ground prosopis pods, 15% barley and 12% dried sardines plus vitamin and mineral additives. The palm date frond silage was prepared by shredding palm fronds to approximately 1-2 cm size. A 3% urea solution was prepared by dissolving commercial fertilizer grade urea in tap water in a large sprayer's tank. Shredded palm fronds was weighed, loaded in a Reel Augie mixer, sprayed with urea solution using a sprayer and mixed thoroughly. The fronds-urea mix was then transferred into a 600-gallon plastic tank and manually pressed as hard as possible to reduce air. The tank was tightly sealed to avoid air entrance to the silage to allow anaerobic processing of fronds and urea mixture. The silage was kept in the tank for 4 weeks at the end of which it was emptied and spread for drying before feeding to animals.

Feed (Rhodesgrass hay, palm frond silage, by-product and commercial concentrates) were analysed for dry matter (DM) by drying in an oven at 60°C until no decrease in weight occurred, and for organic matter (OM) and ash by ashing at 450°C for 12h in a muffle furnace. Triplicate samples of approximately 2 g each were freeze-dried for 4 days for ether extracted (EE) by petroleum ether in a Soxhlet apparatus. Nitrogen was determined by the Kjeldahl method according to the procedures of AOAC (1990). Acid detergent fiber (ADF), cell wall constituent's neutral detergent fiber (NDF) was determined by the methods of Van Soest et al. (1991). Gross energy (GE) was measured using a calorimeter bomb. Analysis for all items was done in duplicates and expressed on dry matter basis.

The animals were slaughtered at the Municipality slaughterhouse in Muscat (Sultanate of Oman) according to routine slaughterhouse methods. The carcasses were kept at 1-3°C for 24h and then the longissimus dorsi from the lumbar (loin) region was removed from the left side of the carcasses and frozen at -20°C until processing. Meat quality-related measurements included ultimate pH, WB-shear force, sarcomere length, expressed juice and color (CIE L*, a*, b*) were determined. The ultimate pH was assessed in homogenates at 20-22°C (using a Ultra Turrax T25 homogenizer) of duplicate 1.5-2 g of muscle tissue in 10 ml of neutralized 5-mM sodium iodoacetate and the pH of the slurry was measured using a Metrohm pH meter (Model No. 744) with a glass electrode. Chilled muscle samples (13×13 mm cross section) for assessment of shear force by a digital Dillon Warner-Bratzler (WB) shear device after being prepared from muscle samples were cooked in a water bath at 70°C for 90 min (Purchas, 1990). Sarcomere length was determined by laser diffraction according to the procedure of Cross et al. (1980/1981). Expressed juice was assessed by a filter paper method, as the total wetted area less the meat area (cm^2) relative to the weight of the sample (g). Approximately 60 min after exposing the fresh surface, CIE L*, a*, b* light reflectance coordinates of the muscle surface were measured at room temperature (25±2°C) using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Japan), with a colour measuring area 1.1 cm diameter. It was calibrated using a Minolta calibration plate (L*=97.59, a*=-5.00, b*=+6.76). The L* value relates to lightness; the a* value to red-green hue where a positive value relates to the red intensity; and the b* value to the yellow-blue where a positive value relates to yellow. The average of two measurements from each sample was

recorded as the colour coordinate value of the sample.

Data were analysed using the GLM procedure within SAS (SAS, 1993), with the model containing items for the four treatments.

RESULTS AND DISCUSSION

All experimental animals including those fed the palm by-products did not have signs of ill health throughout the trial. As g%DM, the by-product and commercial concentrates contained 17 and 19% CP; 24 and 6% ADF; 38 and 18% NDF; 12 and 7% ash; and 18 kj/g gross energy, respectively. The date palm silage and Rhodes grass contained 3 and 7% CP; 58 and 45% ADF; 69 and 76% NDF; 12 and 8% ash; and 21 kj/g gross energy, respectively. The chemical composition of the two concentrates and two roughages used are presented in Table 1. On dry matter basis, the local by-products concentrates and commercial concentrates contained 17 and 19% CP; 24 and 6% ADF; 38 and 18% NDF; 12 and 7% ash; and 18 and 18 kj/g gross energy, respectively. The date frond silage and Rhodes grass contained 3 and 7% CP; 58 and 45% ADF; 69 and 76% NDF; 12 and 8% ash; and 21 and 21 kj/g gross energy, respectively. These diets are cheap and readily available and can be used for feeding sheep.

The main factor determining the quality of meat is its pH, which is related to biochemical processes during the transformation of muscle to meat. Therefore, changes in the pH during the post-mortem period influence the meat quality characteristics. Higher ultimate pH produces dark meat color, reduces storage life and can lead to tougher meat (Chrystall and Daley, 1996). A low plane of feeding can result in chronic nutritional stress, characterized by low reserves of muscle glycogen and increased final pH values in the meat (Bray et al., 1989). Meat ultimate pH values in the present study were not significantly ($P>0.05$) different between the four diet groups ranging between 5.77 to 5.9 (Table 2), and they were within the range for sheep reported by Carson et al. (2001). However, they were higher than those reported by Devine et al. (1993) and Lanza et al. (2003) for ultimate pH values. The ultimate pH value depends on glycogen levels as slaughter. Therefore, lack of differences in ultimate pH values between the four diet groups indicate that there was no effect of the diet, on the muscle glycogen content at slaughter.

Lightness (L*), redness (a*) and yellowness (b*) values of LD muscle were comparable among the four groups (Table 2). Meat color differences can occur due to a direct effect of the diet on the chemical state of the myoglobin on the surface of the meat (Purchas, 1989). Meat color is related to other factors such as ultimate pH, structure and physical state of muscle proteins carcass fatness, age, carcass weight and proportion of intramuscular fat (Carson et al., 2001; Priolo et al., 2001). All these parameters that were similar in the present study across the four diet groups may have collectively contributed towards producing similarity in meat color. The color of the LD muscle in the present study was lower than that reported by Devine et al. (1993), Lanza et al. (2003) and Carson et al. (2001) for sheep, which may be due to differences in ultimate pH. Values of ultimate pH above the isolelectric point of proteins of 5.5 result in an open structured muscle and a greater diffusing of light between the myofibrils of the muscle, which make the cut face of the meat darker (Seideman and Crouse, 1986). The lower L* values of the meat samples from the present study is in line with the expected changes in lightness vales as given by Seideman and Crouse (1986). Moreover, increased pigmentation in meat may be due to a higher iron content in diets rich in forages and concentrates or may be also due to age differences. The low L* and high a* values could be related to higher haem pigment which has been noted to increase with age (Devol et al., 1985).

Water holding capacity is the ability of meat to retain its constituent water when an extraneous force or treatment is applied to it. This property affects the retention of vitamins, minerals and salts, as well as the volume of water retained. Muscles that lose water easily are drier and lose more weight during refrigeration, storage and marketing. Water holding capacity measured as expressed juice was similar between the four diet groups. Sarcomere length was not affected by the experimental diets. Tenderness

variation arises mainly through changes to the myofibrillar protein structure of muscle in the period between animal slaughter and meat consumption. Warner-Bratzler shear force was similar between the four diet groups and it was below 6 kg cm^{-2}, which accounted for acceptable tender samples (Devine et al., 1993). Similarly, Carson et al. (2001) and Lanza et al. (2001) studied feed efficiency of different rations on meat quality of sheep and concluded that sarcomere length or WB-shear force values were not affected. Overall, the values for sarcomere length and for WB-shear force values were higher than those reported by Carson et al. (2001) for British sheep breeds.

CONCLUSIONS

This study indicated that replacing the commercial concentrate and Rhodes grass hay with a more fibrous feed made from local by-products did not produce significant effects on meat quality characteristics.

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Tables

Table 1. Chemical composition of the feeds used in the study (g/100 g DM).

Chemical component	Rhodes grass	Date palm frond silage	Commercial concentrate	By-product concentrate
Crude protein	7	3	19	17
ADF	45	58	6	24
NDF	69	76	18	38
Ash	8	12	7	12
Energy (kg/g)	20.96	19.23	18.32	17.91

Table 2. Effects of date palm frond and prosopis pods on meat quality characteristics of longissimus dorsi muscle in Omani sheep.

Parameter	Diet[1]				SEM	*p*-value
	Group 1	Group 2	Group 3	Group 4		
Ultimate pH	5.77	5.83	5.86	5.90	0.081	NS
WB-shear force	4.46	4.85	4.46	4.95	0.509	NS
Sarcomere length	2.08	2.08	2.00	2.02	0.066	NS
Expressed juice	28.83	30.24	29.12	29.05	0.986	NS
Color *L**	33.73	33.30	35.03	33.79	0.672	NS
Color *b**	11.04	11.10	11.53	11.24	0.425	NS
Color *a**	23.69	24.22	24.43	23.01	0.486	NS

[1] Group1: Rhodes grass hay plus commercial concentrate, Group 2: Urea treated palm frond silage plus commercial concentrate, Group 3: Rhodes grass hay plus commercial concentrate, Group 4: Urea treated palm frond silage plus commercial concentrate.

Applying Submerged Technique to Produce Citric Acid from Dates

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Abstract

Surface fermentation and submerged fermentation by *A. niger* (ATCC 9642) have been carried out to produce citric acid from dates. The submerged technique shows superiority in terms of time reduction as well as yield. The pH drop due to the production of citric acid took ten days in most of the surface fermentation treatments; whereas highest yield of citric acid produced by the submerged fermentation has been obtained after two days. In addition, oxalic did not appear in HPLC assay. To track the formation of citric acid during the fermentation an HPLC and RI detector have been applied successfully through the developed method.

INTRODUCTION

According to the censuses of the Ministry of Agriculture of Oman, the dates production surplus is in the range of 50000-70000 tons and includes unsuitable produce for fancy packing that is usually sold as animal feed at reduced prices. Utilization of the excess to produce derived added value products such as citric acid boosts the economy of the date palm cultivation with its socio-economic adherent role. As revealed by many publications, the main component of all date cultivars is the sugar,which has been found essential for a good production of citric acid. Regarded as the most important organic acid, it is produced in tonnage by fermentation. The widely used technique is the submerged. It is estimated that about 80% of the world production is obtained by submerged fermentation (Al-Abid, 2006). Global production of citric acid in 2004 was about 1.4 million tones estimated by Business Communications Co. (BCC) in a study of fermentation chemical markets (Soccol et al., 2006). Citric acid is widely used in food industries as essential ingredient and to impart a pleasant, tart flavor to foods and beverages. It also finds significant applications in pharmaceutical and chemical industries. For the last 80 years citric has been produced on industrial scale by the fermentation of carbohydrates, exclusively by *Aspergillus niger*, but in recent times by *Candida* yeasts (Kristiansen, 1999). Previous works investigated the application of dates syrup as carbohydrate source to produce citric acid (Roukas and Kotzekodou, 1997; Saad, 2006); however, the fermentation has been carried out by means of a conical flask or surface wise.

The focus of those works was the pretreatment of dibis prior to the fermentation. Throughout the present work, the submerged fermentation of dates juice has been performed in a modern fermentor with controlled parameters.

MATERIALS AND METHODS

Juice Preparation

1 kg of 'Fardh' dates (Precisa PAG 14899, Switzerland) purchased from the local market was placed in 2.5 L of boiling water and left to boil in the water bath (GFL D3006 Germany) for 15 min. The macerated dates were filtered through cheese cloth, and sterilized at 121°C for 15 min (Astell AMP 230). Total soluble solids of the juice were TSS 18°Brix (ABBE, Bellingham and Stanly, UK).

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Analysis of Juice Metal Content

20 g dates juice has been dried at 70°C for 48h then crushed and digested. The mixed acid produced ash of samples was analyzed directly by Philips SP 9 series UK Atomic Absorption Spectrophotometer.

Nitrogen Source

0.2% of ammonium nitrate NH_4NO_3 (BDH) was added to the sterilized juice as a source of nitrogen.

Seed Culture Preparation

100 ml of date juice was inoculated with commercial of *Aspergillus niger* (ATCC 9642), which was cultured in potato dextrose agar according to the OXOID manual and placed in a shaker incubator (Thermoline scientific,UK) at 250 rpm for 48h at 30°C to produce pellets.

Tricalcium Phosphate Treatment

Date juice was adjusted to pH 7.0 (pH-meter WTW inolab Germany), with 1 N NaOH and treated with 2% (w/v) tricalcium phosphate (BDH). The mixture was heated at 105°C for 5 min. After cooling, the mixture was centrifuged (BECKMAN TJ-6 centrifuge, USA) at 4000 rpm for 20 min and the pH of the supernatant adjusted to 6.5 with 1 N HCL to become fermentation ready.

Fermentation Parameters

150-200 pellets were added to the treated and filtered juice and this was placed in the fermentor (LiFlus GX, Biotron, South Korea) (capacity 2 L) and then the pellets were added to it. The fermentor was adjusted to the following parameters: temperature 30°C, agitation intensity (RPM) 500.

Mobile Phase Preparation

1.2 ml of phosphoric acid was diluted with 1 L of deionized water (Millipore water 717 USA) and then placed in a water bath at 70°C until the temperature of the solution reached 60°C and then this was placed in an ultrasonic bath (Ronda EN 631 1/4) for 15 min.

Standards Preparation for HPLC Injection

Standards of some organic acid such as citric acid, oxalic acid, tartaric acid, and maleic acid were prepared by taking 0.05 g (Precisa XB220,Swizerland) of each of them and then diluting separately with 50 ml of the mobile phase then filtered through filter paper, solid phase extraction through Sep-Pak Cartridges (Waters, USA) and then injected in HPLC (Waters 600E) column (Shodex KC-811 with guard, Waters) to make calibration peaks (Waters Millennium software).

Sample Preparation for HPLC Injection

1 ml of the fermentation product was diluted with 50 ml of the mobile phase and then filtered similar to that of standards and then injected in HPLC to determine the concentration of citric acid. The progress of the fermentation was pursued through daily analysis of citric acid concentration in the fermentation broth by an HPLC-RI detector (Waters 2414).

RESULTS AND DISCUSSION

Surface Fermentation

Four trials have been carried out to achieve the highest production of citric acid. Results obtained are shown in Figures 1 to 4. Generally, a decrease of pH values reveals ascending citric acid production along with time progress. This decrease takes place

gradually in treatment I and II, whereas results of treatments III and IV show a significant pH drop in the first 3-4 days of the fermentation (Figs. 1-4). This could be due to the applied tricalcium phosphate, which acts as chelating reagent. Thus, treatments III and IV can be terminated in the first weak. In the case of treatment I and II the pH decrease starts to become flat during the tenth day of fermentation. To determine the end phase, the fermentation experiments were run three days extra more without any sign of pH change. As we know, sugars as well as other nutrients are fermentation substrate; therefore TSS values are lessening with the time proportional to the pH decrease. The above results indicate that the applying of chelating agent enhances the fermentation. However, the fermentation progress proceeded gradually.

Submerged Fermentation

Numerous trails have been carried out to study the production of citric acid from 'Fardh'. The dates juice was inoculated by *Aspergillus niger* ATCC 9642 (pellets). Nutrient ammonium nitrate (0.2%) was applied as well as the metal chelating agent tricalcium phosphate. After many trials we found that 500 rpm was the most appropriate rotation speed to distribute the pumped air and control the dissolved oxygen, also to keep the fungi functional. The progress of the fermentation has been pursued through daily analysis of citric acid concentration in the broth by HPLC-RI detector. Methodology of the detection such as sample preparation, selection and conditioning of the separation column as well as other conditions has been developed in house. The chromatograms show excellent peaks resolution indicating the success of the applied analyzing technique.

The chromatogram (Fig. 5) shows reasonable yield of citric acid after seven days of fermentation. However, the yield was higher at the second day of the fermentation as revealed in Figure 6. Accordingly, three days would be required to accomplish the highest production level of citric acid, noticeably shorter than the surface method. It is a well known fact, that the various minerals of the substrate affect the citric acid fermentation. The analysis of the metal content shows that the applied dates juice contains Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}. The concentrations found are shown in Figure 7. Interesting is the concentration of zinc, which is considerably high (0.696 mg/L). It is supposed to affect the accumulation of citric acid. Low concentrations of zinc in the fermentation medium are generally favored in most citric acid production media. At high zinc levels (about 2 μM) the cultures are maintained in growth phase, but when the medium becomes zinc deficient (below 0.2 μM = 0.0131 mg) growth is terminated and citric acid accumulation begins (Yigitoglu, 1992). It has been reported that zinc deficiencies promote citric acid production. In addition, zinc plays a role in the regulation of growth and citrate accumulation.

The addition of tricalcium phosphate was designated to bind all these metals according to previous work (Roukas and Kotzekidou, 1997; Saad, 2006). Furthermore, many trials have been carried out to optimize the fermentation parameters such as agitation intensity (RPM), supply of air, initial Brix, pH of the juice, quantity of vegetative inoculums and incubation temperature. This controlled fermentation reveals an important sign of success namely the disappearance of oxalic acid. The rapid decrease of the pH may be the first explanation since the oxalic acid producing enzyme - oxaloacetate hydrolase - becomes at low pH very low active. As a second reason, the applied strain could be lacking oxaloacetate hydrolase and glucose oxidase (Kristiansen, 1999). However, by-products from citric acid fermentation should be minimized through regulation of the fermentation parameters. The submerged technique should be considered in any prospective project to produce citric acid from dates despite the sophisticated installations and rigorous control. Nevertheless, it presents several advantages such as higher productivity and yields, lower labor costs, lower contamination risk and manpower (Al-Abid, 2006).

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Figures

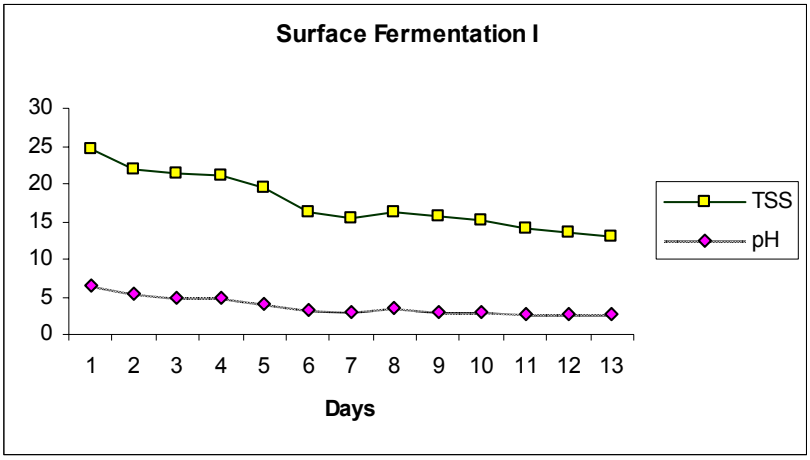


Fig. 1. Treatment I: without ammonium nitrate (0.2%) as a source of nitrogen and without TP (tricalcium phosphate) as chelating agent. Results shown in the graph below.

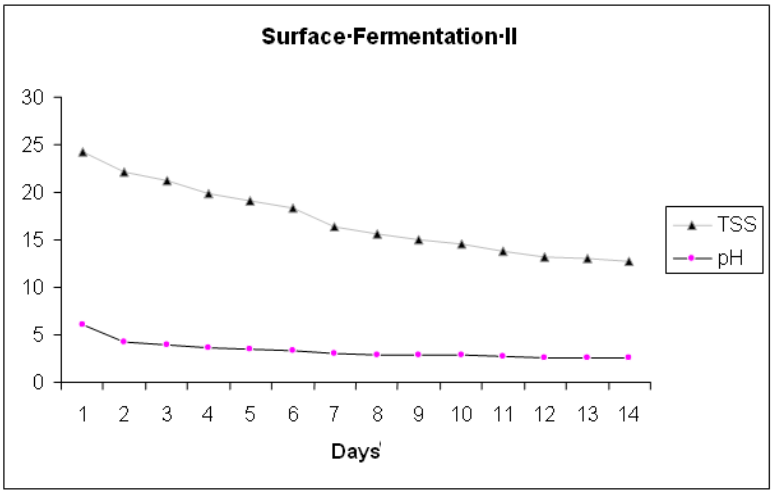


Fig. 2. Treatment II: ammonium nitrate (0.2%) as a source of nitrogen without TP.

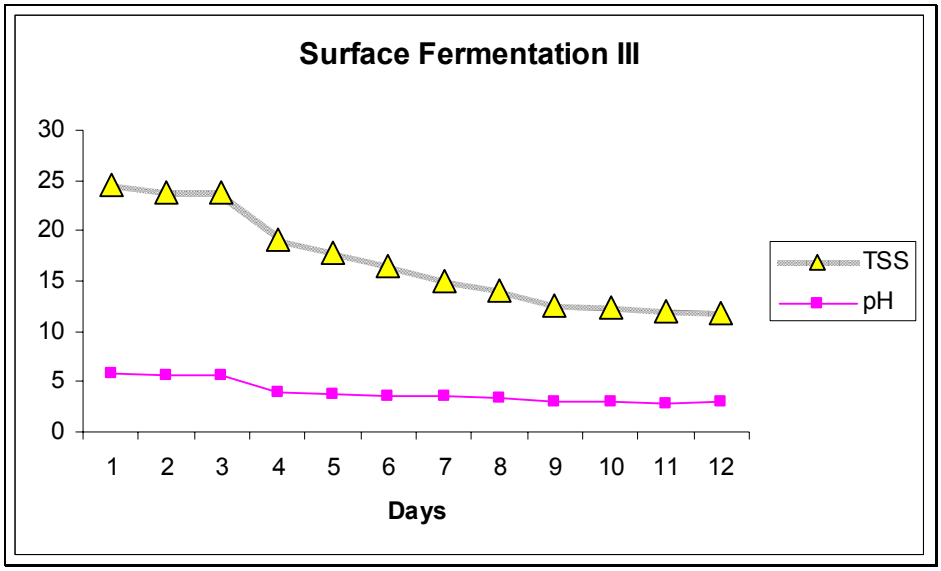


Fig. 3. Treatment III: ammonium nitrate (0.2%) as a source of nitrogen with TP (without centrifuge).

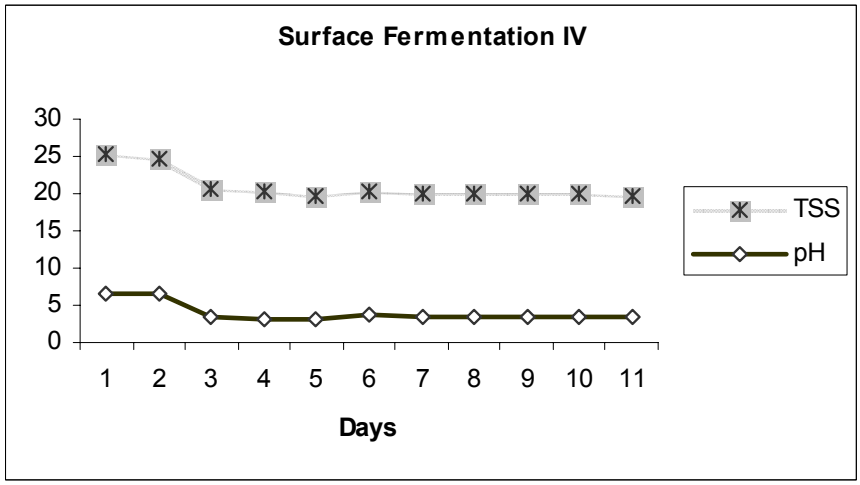
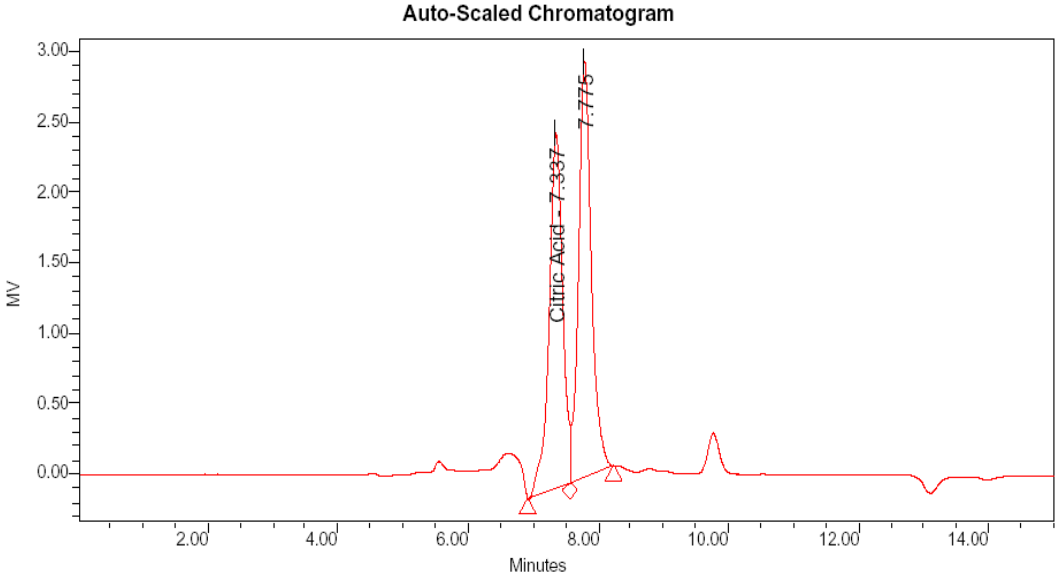


Fig. 4. Treatment IV: ammonium nitrate (0.2%) as a source of nitrogen with TP (without centrifuge).

SAMPLE INFORMATION

Sample Name:	ferm sample D7.28.2008 Sep	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	28-Sep-08 11:56:04 AM
Vial:	24	Acq. Method Set:	Organic Acids_Set
Injection #:	1	Date Processed:	28-Sep-08 12:11:18 PM
Injection Volume:	5.00 ul	Processing Method:	Organic Acid_demo
Run Time:	15.0 Minutes	Channel Name:	410
Sample Set Name:		Proc. Chnl. Descr.:	



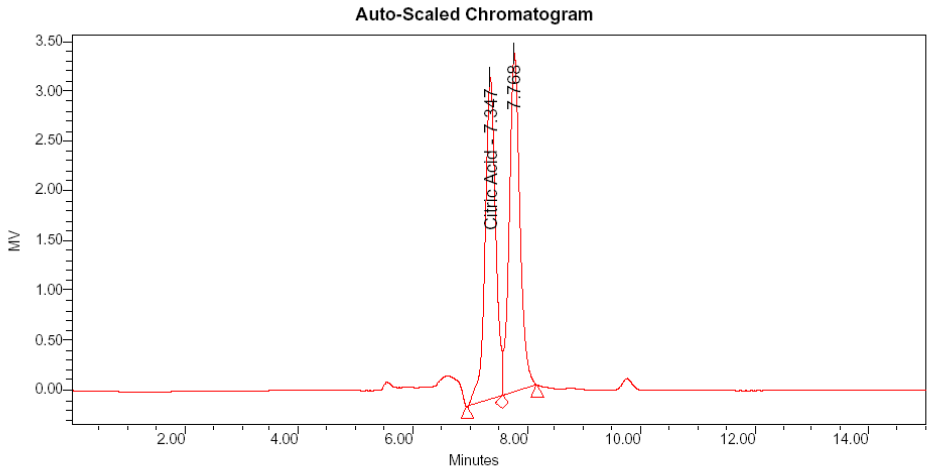
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Oxalic Acid	5.878				
2	Citric Acid	7.337	36712	2528	91.818	ppm
3		7.775	40330	2965		

Fig. 5. Citric acid chromatogram at the 7thday of the fermention (Exp. 10).

SAMPLE INFORMATION

Sample Name:	ferm 2D 23/9/08	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	23-Sep-08 11:32:53 AM
Vial:	19	Acq. Method Set:	Organic Acids_Set
Injection #:	3	Date Processed:	23-Sep-08 11:48:06 AM
Injection Volume:	5.00 ul	Processing Method:	Organic Acid_demo
Run Time:	15.0 Minutes	Channel Name:	410
Sample Set Name:		Proc. Chnl. Descr.:	



Peak Results

	Name	RT	Area	Height	Amount	Units
1	Oxalic Acid	5.878				
2	Citric Acid	7.347	42080	3244	102.057	ppm
3		7.768	44032	3400		

Fig. 6. Citric acid chromatogram at the 2nd day of the fermentation (Exp. 10).

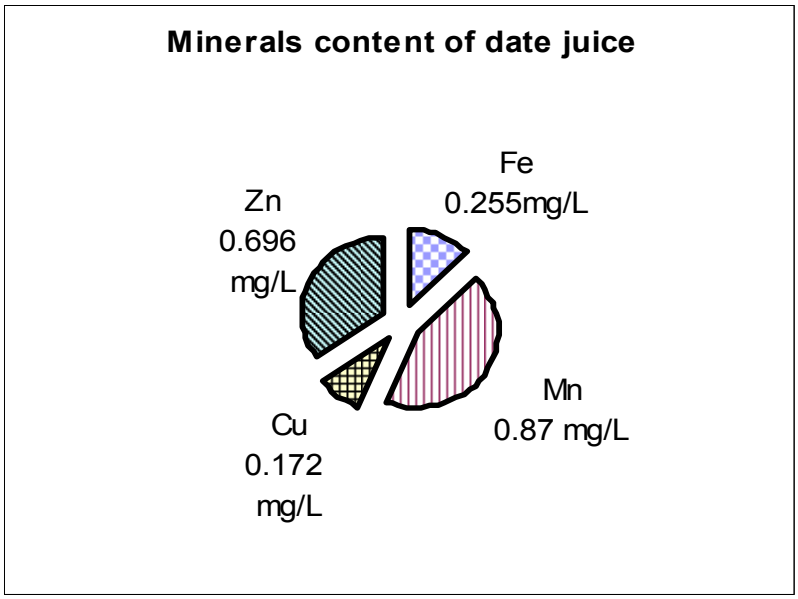


Fig. 7. Minerals of date juice.

UAE Standards of Palm Dates and Its Products as a Main Item of Small Enterprises

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Represents: Emirates Authority of Standardsation and Metrology (ESMA)
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WHO IS ESMA?
Federal Law no. 28 of 2001, Article 4.
ESMA is the sole authority for Quality, Metrology, and Standards in the UAE.

Esma Goals
1. Supporting safety and protect the consumer, economy, and environment by ensuring that services and products (both locally manufactured or imported) are in conformity with the UAE standards.
2. Protecting national economy by assisting the national industry to enter international competition and global markets.
3. Provide excellent services by Implementing worldwide leading practices and certified internal processes for dealing with customers.

Field of Activites
- Standard: issuance and revision.
- Product certification and quality: run national scheme (ECAS) and national quality mark (QM).
- Accreditation: accredit conformity assessment bodies (labs and certification bodies).
- Legal metrology: regulate and monitor the implementation.

LEVEL OF STANDARDS SHOWN IN FIGURE 2

Examples of UAE Technical Regulations Established in 2008
1. Food label.
2. Expiration dates.
3. Loose dates
4. Prepackaged whole dates.
5. Date syrup.
6. General requirements for transportation of chilled and frozen foods.

UAE STANDARDS OF FOOD PRODUCTS

Fields of UAE Standards and Tech. Regulations of Foodstuffs
1. Baby foods.
2. Cereals.
3. Dairy.
4. Drinking water.
5. Drinks.
6. Feedstuffs.
7. Fish.
8. Food additives.
9. Food containers.
10. Fruits and vegetables.
11. Generals.
12. Meat.

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13. Oils.
14. Special dietary foods.
15. Spices.
16. Sugars and confectionaries.
17. Tea and coffee.
18. Tobacco.

UAE standards for date products
No.656 Prepackaged whole dates.
No.1813 Date (debis) syrup.
No.1869 Date paste.
No.5003 Loose date.

Complementary Standards as Guidelines for Production and Handling of Dates
- No. 9: Labeling of prepackaged foods (Fig. 3).
- No. 150: Expiration periods at food products.
- No. CAC GL 1: General guidelines on claims.
- No. CAC GL 23: Guidelines of nutrition labeling (Fig. 4).
- No. CAC GL 32: Guidelines for the production, processing, labeling and marketing of organically produced foods.
- No. 168: Conditions of storage facilities for dry and canned foodstuffs. Figure 5
- No. CAC 106: General standard for irradiated foods

SMALL ENTERPRISES

Definition
The definition of small enterprises is different from one State to another, and usually depends on the number of employees and determines the value of capital.
- Small enterprises, including:
  - Commodities and products.
  - Services.
  - Activities.
- Commodities and products may also include:
  - -Traditional products.
  - -Products for general consumers.
  - -Production requirements.

Elements to Be Concidered for Small Enterprises
- Specialization.
- Quality.
- Rehabilitation.
- Knowledge, information and market research.
- Use of modern techniques.
- Take advantage of available services.

- **Specifications**

Standard specifications have covered all of these elements in the specification of an independent or within the standard specifications for each commodity to be indicative or guidelines of a mechanism for small enterprises as follows:
- Standards of the requirements for production places.
- Standards of the product.
- Standards for the methods of transport, storage and trading.
- Standards for guidelines and good practices.
- Standards for raw materials and production requirements.

• **Quality production**

The definition of quality in a simple meaning: the conformity of a product with the specifications laid down on it, or the extent to which the product covers the consumer demands, or suitability of the product for use in a satisfactory manner.

According to the definition of ISO Standard no. (8402) is:

"Group elements and characteristics of a product or service to respond to the needs of the stated or implied".

The different level of quality required in this case depends on:

- The type of consumer in terms of both level of life or consciousness or cultural traditions or religious beliefs.
- Level of economic development.

For all these factors, the developing of national standards should be important.

Examples of raw materials and evironmental industries, traditional and value-added products and comparitive advantages as a componant of small enterprises:

1. Palm and its products:

The most important raw materials and environmental industries and the traditional desert environment are the palm products, mainly palm dates and its fruits (dates) as follows:

- Products of palm fronds and palm roots as baskets (Fig. 6).
- Arum products, including ropes (Fig. 7).
- Palm trunks used in construction (Figs. 8 and 9).

2. Date and date products:

- Prepackaged whole dates (Fig. 10).
- Loose date (Fig. 11).
- Date paste (spread).
- Date sheets (spread) (Fig. 12).
- Date pickles (Fig. 13).
- Diced dates (Fig. 14).
- Date pits (Fig. 15).

STANDARDS FOR DATE AND DATE PRODUCTS

prepacked whole dates in brief / uae.s/gso 656 / codex standard for dates (world-wide standard)

This standard is concerned with whole dates (*Phoenix dactylifera* L.) in pitted or un-pitted style, packed and ready for direct human consumption and its sampling methods. It does not apply to other forms of dates such as pieces or smashed dates or dates for industrial purposes or used as feed.

1. Definitions

- Dates: the product prepared from sound suitably ripe fruit of the date tree. (*Phoenix dactylifera* L). They may be washed and/or pasteurized, and may be dried or hydrated to adjust the moisture content.
- Pitted dates.
- Cane sugar dates: dates in which most of the sugar content is in the form of disaccharide sugar (sucrose) such as 'Sukkari' and 'Deglet Noor' and 'Deglet Bedhi'.
- Invert sugar dates: dates in which most of the sugar content is in the form of invert sugar (glucose and fructose) such as 'Berni', 'Khasaab', 'Fardh', 'Mabsoli', 'Naghal', 'Hamzal', 'Barhi', 'Khalas', 'Khadrawy' and 'Zahiry'.
- Pressed dates.
- Loose dates.
- Strands.
- Blemished dates.
- Damaged dates (for unpitted dates).
- Unripe dates.
- Unpollinated dates.

- Dirty dates.
- Dates having insects and mites damage or contamination.
- Souring dates.
- Mould dates.
- Decay dates.

2. Classification

Dates shall be classified according to the following:

- Sugar types
  - Cane sugar varieties such as: 'Deglet Noor', 'Deglet Bedhi', 'Meskany' and 'Anbarah'.
  - Invert sugar varieties such as: 'Berni', 'Khasaab', 'Fardh', 'Mabsoli', 'Naghal', 'Hamazal', 'Barhi' and 'Khalas'.
- Styles
  - Unpitted dates.
  - Pitted dates (unstuffed or stuffed).
- Size of date (optional). Dates are classified into three sizes: small, medium and large. The number of pitted and un-pitted dates for each size per 500 grams shall be as indicated in the following Table:

Size grading of dates		
Size of date	Number of dates in each 500 g	
	Pitted dates	Unpitted dates
Small	More than 110	More than 90
Medium	90 to 110	80 to 90
Large	Less than 90	Less than 80

3. Main Requirements

Whole prepackaged dates shall meet the following requirements:

- They shall be free from live insects, their eggs, larvae and excretions.
- The raw materials used in production shall comply with their relevant standards.
- It is allowed to add the following substances; provided that they comply with their relevant standards:
  - Glucose syrup, sugars, flour, vegetable and tahinia (sesame paste) oils.
  - Natural flavors, in case of filled dates.
  - Glycerol or sorbitol , in case of dates covered with glucose syrup.
- The average weight of any unit of dates shall not be less than 4.0 g for pitted dates and not less than 4.75 g for unpitted dates.
- The moisture content shall not be more than 22% by weight for cane sugar varieties and not more than 26% by weight for invert sugar varieties.
- Pitted dates may be filled with coconut, almonds or any other type of nuts.
- The metal impurities (such as metallic parts, nails, metallic fillings, etc.) shall not be more than 1 g/kg.
- The maximum allowable defects shall be as follows:
  - A total of 7% by count blemished dates.
  - A total of 6% by count damaged dates, unripe dates and unpollinated dates.
  - A total of 7% by count dirty and contaminated dates and dates having insects and mites damage.
  - A total of 1% by count souring dates, mould dates and decayed dates.
- Their production shall be according to the GSO standard mentioned. Pesticide residues shall not exceed the limits mentioned in the GSO standards.

4. Labelling

Without prejudice to what is mentioned in GSO standard mentioned in 2.1, the following information shall be declared on the labels:

- Name of the product:
  - "Dates", "Dates coated with glucose syrup" or "Filled dates".
  - "Unpitted" or "pitted" in addition to the size (small, medium or large).
  - The varietal type as "cane sugar varieties" or "invert sugar varieties".
  - The name of the variety such as 'Deglet Noor', 'Sukkari', 'Berni', 'Khasaab', 'Fardh', or others, may be declared.
  - Whether it is "pressed", "loose" or in "clusters".
- Name of the additives (if used).
- The stuff type or coating material (if any).
- Date of packaging and expiry in a non-coded manner (month and year), according to the GSO standard.
- Country of origin

Any nutritional information may be declared on the label provided that it conforms to the requirements mentioned in the GSO standard.

Date debis (syrup) in brief / UAE.S/GSO 1813 / Scope and Field of Application

Date debis (syrup) is extracted from whole mature date fruits (*Phoenix Dactylifera* L.) in different ways.

1. Main Requirements

Debis shall meet the following requirements:

- Dates which debis is made of shall comply with Saudi standard mentioned.
- Debis shall be homogenized, viscons liquid, uncrystallized.
- Debis shall be free of insects, their parts or mites excreta.
- Debis shall be free from artificial substances.
- Soluble solids shall not be less than 70% (m/m).
- pH shall not be less than 4.5.
- Shall be free of fermentation and life micro-organisms causing hazards to health and their products that enable to grow in storage conditions.
- Metallic elements shall not exceed the following limits in the final product:
  - Arsenic 1.0 ppm
  - Copper 2.0 ppm
  - Lead 1.0 ppm
- Total sugars shall not be less than 68% (m/m).
- Moisture content shall not be more than 27% (m/m).

2. Packaging, Transportation and Storage

- Packaging: the product shall be packed in hygienic and impermeable to moisture container and well sealed according to the GSO standard mentioned.
- Transportation: shall be carried out in such a way as to protect packages from mechanical damage, contamination and direct sunshine.
- Storage: the packages shall be kept in stores that are clean, well-ventilated, and far away from sources of heat, moisture, and contamination, according to the GSO standard mentioned.

3. Labelling

Without prejudice to the requirements mentioned in GSO standard (item 2.1) the following shall be considered:

- Date of production.
- Type extraction method.
- Name of date fruits extraction.
- Concentration of soluble solids % (m/m).

Standard of date paste in brief / UAE.S/GSO 1869

Date paste: a simple additional grinding operation will turn the macerated date into date paste. The principle is the same as for prepared minced meat and the fineness of the product can be regulated by the use of different size holes in the discs. Refrigeration may be required to prevent possible fermentation. Date paste is also produced commercially in Gulf area and used for bakery products.

1. Scope and Field of Application

This standard is concerned with whole date paste, packed and ready for direct human consumption.

2. Definition

Date Paste: food product prepared from the sound fruits and uniform in color and appropriate stage of maturity of the fruits of date palm (*Phoenix dactylifera* L), washed and removed pits, the intervention in the manufacture of biscuits, sweets, pastries and ice cream and may be mixed with other food products such as sesame or peanuts or nuts.

3. Requirements

Date paste shall meet the following:

- -Soft, homogeneous in color, texture, and not by blackening or stiffness or change in the smell and taste.
- Free of whole or broken pits and cones and foreign parts.
- Does not allow the addition of any coloring matter.
- To be free from fermentation and decay.
- The moisture content shall not exceed 20%.
- The metallic impurities content shall not exceed 2 g/kg.

Standard of Loose (Unpackaged) Dates in brief / UAE.S/GSO 5003 (Fig. 15)

1. Definition

Dates that are brought to the consumer without packaging

- To be in the appropriate stage of maturity (stage of dates).
- Does not increase the proportion of moisture in the dates of 20% by mass.
- Metallic impurities, such as cutting and metal parts, nail and metals powder should not exceed 1 g/kg.
- The maximum allowable for each sample of the defects should be as the following:
  - Damaged dates 7%.
  - Unripe dates, unpollinated dates 6%.
  - Dirty dates, dates having insects and mites damage or contamination 7%.
  - Souring dates, mould dates, decay dates 1%.
- Dates to be free from pathogenic microorganisms and also not containing material resulting from these neighborhoods may make them in quantities harmful to health, Ref.: Inter. Standards (Codex).
- Should not exceed the limits of pesticide residues allowed in the Gulf standard specifications to be adopted by ESMA.

PROPOSED NEW STANDARDS FOR OTHER DATE PRODUCTS IN UAE - HOW CAN THE STANDARDS BE A COMPONENT OF GULF BASE FOR SMALL ENTERPRISES?

- Date juice.
- Date sheets (Spread).
- Date pickles.
- Palm fronds.

Date Juice

Dates are different from other fruits from which juices are obtained by pressing (e.g., citrus, apple, grapes and mangoes) in that their soluble solids are too concentrated to be pressed out and syrup (dibs) is produced as an incidental by-product. To produce date juice, water has therefore to be added to dissolve and dilute the soluble solids of the date, after which the non-soluble solids are separated out.

Date Sheets (Spread)

Despite the success of numerous tests for the production of dates sheets, this product has not found its way into production on industrial scale pattern on the chip apricot (apricot), which has a large production in the Gulf countries despite the installation of high nutritional value of the same dates chips or sheets. No different segments of the production processes of a paste of dates for many segments of apricot paste, except that the percentage of sugar is higher and there is less acidity than in the apricots.

There is no doubt that the completion of the issuance of a Gulf standard for Gulf date paste, which lasted for more than a year is a step on the way to produce dates chips or date sheets on an industrial scale within small enterprises.

Diced Dates

Diced dates which are cut up rather than extruded macerated dates. The operation is performed in a dicer, which can be set to produce pieces of about 3/16" to ½". They are also coated with dextrose or oat flour to keep the pieces separate.

ESMA IN BRIEF

Definition of Small Enterprises

It includes: Commodities and products, services, activities. The products may include: traditional products, products for the general consumers, raw materials.

The elements Considered for Small Projects

Specialization, quality, training, information and market studies, uses of modern technologies, uses advantage of available services.

Gulf Standards as Main Element for Small Enterprises Base

The requirements of all these mentioned elements have been addressed within general standards or specific standards for each commodity to be the guidelines for a mechanism of small enterprises – as the following:

- The requirements of manufacture sites.
- Standard of product.
- Standard of transportation sStorage and handling.
- Standard of labelling.
- Standard of raw materials and ingredients.

Examples: (palm trees and its products) as raw environmental materials, traditional industries and value-added products and the comparative advantages as a component of small enterprises.

Products of palm are the most important raw materials of environmental and traditional industries in the UAE and the Gulf area. At the forefront of dates, as fruit itself but dates are used as a component within the many other food industries such as biscuits, sweets and pastries, and others. The most important by-products of the palm are plaited palm leaflets, palm roots, palm fronds, palm arum, palm trunk used in construction.

1. Date and Its Products, as Traditional and Small Industries.

- Gulf Standards for dates.
  Prepackage whole dates - loose dates - date paste - date syrup.
- Technical guidance for the manufacture dates in Gulf Standards.

- Date industries.
  Packed dates - loose dates - date syrup - date juice - date spread (paste) - date shreds - date pickles and date chutney.

2. By-Products of Palm Trees. Date pits products - clusters - palm trunk - palm fronds - plaited palm leaflets - palm arum.

3. Proposed Drafts as UAE Specifications. Date juice - date shreds - date pickles - palm arum ropes - palm fronds for traditional products.

Figures

Fig. 1. Date products.

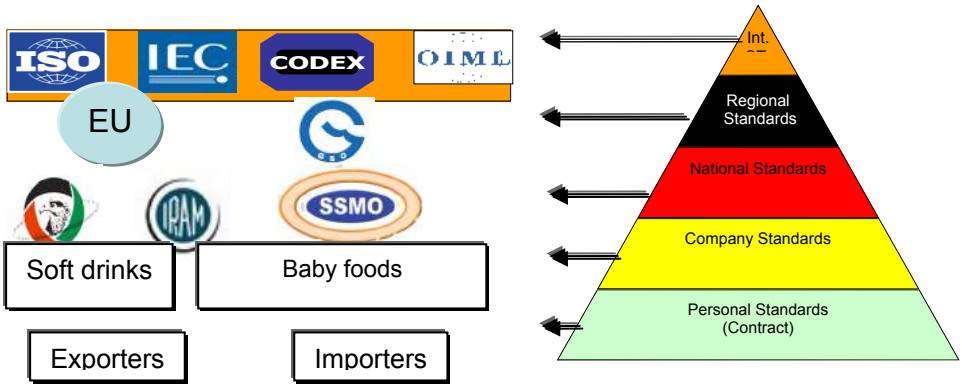

Fig. 2. Level of standards.

Fig. 3. Labeling of prepackaged foods.

Fig. 4. Nutritional labeling.

Fig. 5. Storage facilities for dry and canned foodstuffs.

Fig. 6. Products of palm fronds and palm roots as baskets.

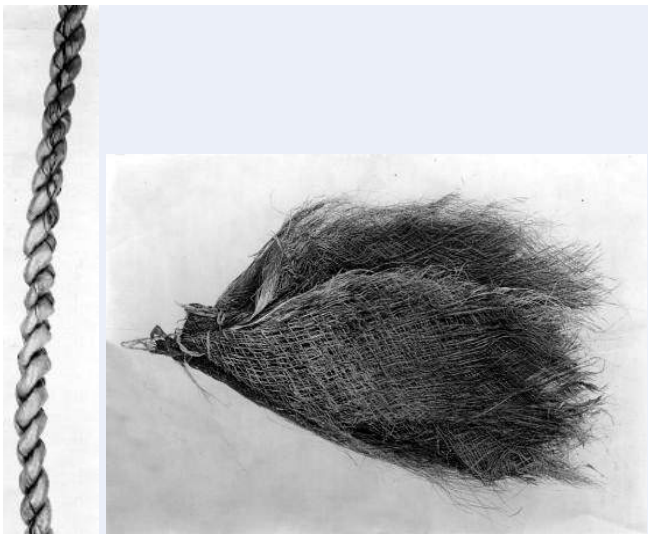

Fig. 7. Arum products.

Fig. 8. Palm trunks.

Fig. 9. Corresponding small enterprises.

Fig. 10. Prepacked whole dates.

Fig. 11. Loose dates.

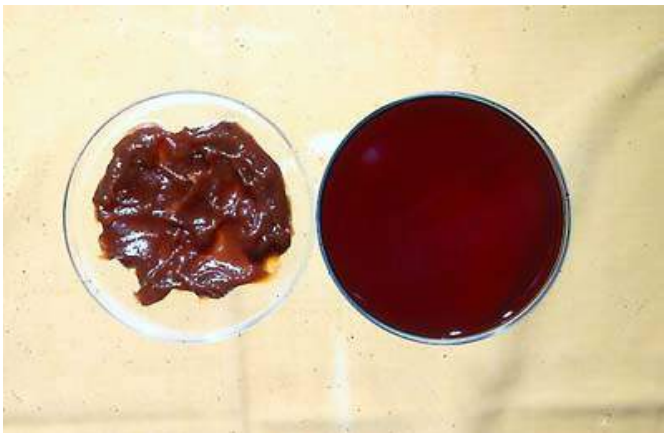

Fig. 12. Date spread compared to date syrup produced from the same raw material.

Fig. 13. Date pickles.

Fig. 14. Diced dates.

Fig. 15. Date pits.

A Comparative Study for Competitiveness of Dates from the Kingdom of Saudi Arabia and the United Arab Emirates in the World Market

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Keywords: world trade, revealed comparative advantage, competitive advantage, penetration ratio

Abstract

Although the KSA and UAE are the third and the fourth largest world producers of dates, their exports face strong competition in the international market. Dates from both countries suffer from low prices. This has a negative impact not only on the export level but also on the farmers, consequently adversely impacting the balance sheet of the agricultural sector and the Government financial plan. Dates represent the main crop that can help the KSA and UAE decrease the deficiency of the balance sheet of the agricultural sector.

The results show that dates are the most important export crop from the KSA and UAE in the foreign market. Its export value and quantity have increased from year to year. Results show that the KSA and UAE marketing systems of date have a good efficiency and are competitive in the Arabic market but have less competitive ability in the European and American market. The result shows that the competitive ability of the Kingdom's dates is stronger than Emirate's dates in general in the Arabic markets, especially in Kuwait and Yemen. Because most of the production of dates in both countries are dried varieties, their production is more suitable for Arabic and Islamic countries. Both the KSA and UAE occupy lower ranks in the competitive ability in European markets. Both the KSA and UAE have a very limited exported quantity of dates to European countries and America. The KSA was the fifth for its market share and rate of penetration in the British market after Israel, Tunisia, Iran and Pakistan. In the French market only about 5 tons are exported Saudi dates. The production of dates for export must be according to special favored characteristics of foreign consumers' needs. So it is important to focus on consumer's needs of fresh, dried semi dried or manufactured dates products especially for promising countries such as Islamic, Asian, American and European regions.

The most important competing countries for KSA dates are the UAE, Iran, Pakistan and Tunisia. The results show that the KSA has a price advantage compared to Tunisian dates, but it does not have a price advantage for the UAE, Iran and Pakistan's dates. The UAE play an important role for exporting and re-exporting dates especially for Asian countries, but it is important to pay more attention to export to European and American countries by activating a complementary strategy with the KSA.

The results of a case study from the KSA, which included 150 farms, 35 traders in the whole market and 20 export companies, confirm the results of previous analysis of secondary data and insures the necessary cooperation of marketing and production for building a complementary strategy between the KSA and the UAE. This strategy must include fresh, dried and manufactured dates and depend on an efficient local and international database.

In reality manufacturing sector of dates in both countries, needs to be more active, especially, for creating new products of dates as an alternative of overseas products. This strategy has to improve technical capabilities, environmental issues,

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quality management. Therefore, it is important to build a suitable complementary strategy for production and marketing of dates from the KSA and the UAE in local and international markets for removing the negative effect of unorganized competition and decrease the surplus in local markets by opening new markets overseas in the future. This will increase the export price and positively impact not only the export sector but also the farmers, consequently moreover on the Government financial plan for agriculture. Dates represent the main crop that can help the KSA and the UAE decrease the deficiency of the balance sheet of the agricultural sector.

INTRODUCTION

Export problems are most important priorities facing all countries. Developed and developing countries after GATT and the free trade system established their strategies for improving their competitive abilities in the world markets.

The Gulf region of the Middle-East which comprises six countries viz. the Kingdom of Saudi Arabia (KSA), the United Arab Emirates (UAE), the Sultanate of Oman, Kuwait, Qatar and Bahrain, accounts for nearly 30% of the global date production. The KSA and the UAE are the regions' top producers. During the last two decades, there has been a significant increase in the area under date palm in these two countries. Producers of dates in both countries suffer from low dates price as a result of surplus of supply. It is important to study the marketing competitive ability in the KSA and the UAE because they are considered the largest Arab dates exporters. It is also important to study their competition ability compared with other competing countries in the world market in order to estimate the competitive ability and possibility of improvement in the future.

The Gulf region of the Middle-East which comprises six countries viz. the Kingdom of Saudi Arabia (KSA), the United Arab Emirates (UAE), the Sultanate of Oman, Kuwait, Qatar and Bahrain, accounts for nearly 30% of the global date production (http://en.wikipedia.org/wiki/Date_(fruit)#Fruit). Although the KSA and the UAE are the third and the fourth largest date producers in the world, they face many problems during production and marketing. Moreover, they face strong competition in the world market. Increasing date exports can be of help to the KSA and the UAE to decrease their deficit of the agricultural balance. Many studies refer to the increase of competition in the global fruit markets (Murray, 1998), There are significant changes in the structure and strategy of the private fruit export company sector.

This study aims to analyse the competitive situation of both Saudi and Emirate dates compared with the most important competing countries in the world market.

In addition, it determined the strength and weakness of KSA and Emirate dates in the foreign market. Moreover, it determined the promising markets for the KSA and the UAE dates in the future. Furthermore, the possibilities of improving marketing efficiency in both countries in the future are determined. This will positively affect farmers, traders, manufacturers and the agricultural balance of both countries.

METHODOLOGY

The study depends on descriptive and statistical analysis such as trends, regression, anova analysis and LSD test. In addition to some important economic indicators the competitive abilities of KSA and UAE dates in the most important foreign markets are analysed. Revealed comparative advantage was used, market share, price competitiveness and penetration ratio. The study depends on secondary data from local level such as the agricultural ministry and the economic and planning ministry and international level such as www.faostat.org and www.comtrade.un.org. In addition to primary data, where the questionnaire of KSA includes 150 farms, 35 traders in the whole market and 20 export companies were selected randomly.

Important Competitive indicators

1. Revealed Comparative Advantage. (Research information sector, 1998). According

to Balassa the revealed comparative advantage is estimated as follows:

$$RCA = (Xji/ Xwi) / (Xjt/ Xwt) \quad (1)$$

Where, RCA: index number for revealed comparative advantage; Xji: export value of product i for the country j; Xwi: export value of product i in the world; Xjt: total export value for the country j; Xwt: total export value for the world.

It is possible to calculate this number for one agricultural crop, or for total agricultural crops or for group of agricultural crops. Increasing this number to 100 means that there is revealed comparative advantage for exporting the crop from the country. Decreasing this number relative to 100 means that there is no revealed comparative advantage for exporting the crop from the country.

2. Market Share Indicator. (US Agency for International development, 1998). Market share is one of the most important indicators of competitiveness. The bigger the number of the market share the bigger of competitiveness for the country.

$$MSHji = (Xjci/ Mcwi) *100 \quad (2)$$

Where, MSHji: market Share for the country j of crop i; Xjci: export from country j to country c of crop i; Mcwi: total import for the country c from the world of crop i.

3. Competitive Price. (Research information sector, 1998). Competitive price is affected by many factors such as relative price, reliability and marketing efficiency. The most significant previous factor on price competitiveness is relative price. Competitive price can be found by obtaining: firstly: the price ratio between the country and competitive countries for exporting one crop. This occurs by calculated the percentage between the averages of crop prices in the country to averages of export prices for every country. The higher the competitiveness price indicator over one the more competitiveness for the country for exporting the crop.

$$PAj = pc/pe \quad (3)$$

Where, PAj: percentage of average export price of the most important competitive countries to average price of determined country; Pc: average export price of the most important competitive countries; Pe: average price of determined country.

Secondly, by estimating the relative situation for the country comparing the competitive countries as the following equation:

$$RAj = PAj – PA\ min / PA\ max – P\ A\ Min \quad (4)$$

Where, PAj: relative situation for determined country comparing to export price of other countries; PA max, PA Min: maximum and minimum for the percent between average export price for the most important competitors to the average export price for every country of competitors.

The value of relative situation of the competitive price competitiveness indicator is between zero and one. The bigger the value of relative situation of competitive price is the better the competitiveness of the country.

4. Market Penetration Ratio. Market penetration ratio is an important indicator of competitiveness. It is described as the percent between import country (c) of crop (i) from country (j) (Market Penetration Ratio will be for country (j) to revealed consumption of crop I as the following:

$$MPRjci = Mcij / (Qci + Mci – Xci) \quad (5)$$

Where, MPRjci: Market Penetration Ratio of country j for crop i in country c; Mcij: import of country c from country j of crop i; Qci: production of crop i for country c; Mci:

total import of i for country c; Xci: export of country c of crop i.

The value of the penetration ratio is between zero and one. The more increase in the value the more entry becomes easily for this market.

RESULTS AND DISCUSSION

Area and Production of Dates in the Gulf Region

Date is considered the most important crop in Gulf countries. Date is a source for gulf countries of food security. In the last decade, the KSA and the UAE pay more attention to date palm cultivation. Scientific research studies related to date palm are important priorities of Gulf countries (Erskine, 2006).

It is clear from Figure 1 that the increase of the palm dates area differs from one country to another in the Gulf region. The UAE achieved the biggest increase for area of palm date by about 740% from 22,156 to 186,000 ha to become the largest producer. The KSA achieved about 136% increase in the area from 64,073 to 151,000 ha. Followed by Kuwait, Qatar, Oman and Bahrain by about 480, 61, 26 and 9%, respectively. It is clear from Figures 1 and 2 that the UAE has achieved a shift of date's area and production. The UAE will increase the production of dates in the few next years as a result of new production of the cultivation area. Therefore, it is necessary for the KSA and the UAE to organize production and marketing of dates in order to decrease the possibility of a negative impact of competition between the two countries in the local and international market.

Although the KSA and the UAE increased the cultivated area during the last decade, they face many problems during the production and marketing process. The most important production problems are low level of production efficiency, infection with diseases, insufficient clever workers of palm dates. On the other hand, the KSA and the UAE suffer from many marketing problems as many developing countries. Marketing of dates in both countries suffer from low level of efficiency and lack of information.

Indicators of Competitiveness for the KSA and the UAE

This part deals with applied indicators of competitiveness for KSA and UAE's dates and comparing it to the most important competitors.

1. Revealed Comparative Advantage

Revealed Comparative Advantage for KSA and UAE's Dates. The high value of revealed comparative advantage for KSA and UAE's dates is clear from Table 1. It was above one during the three periods of the study (1998-2005). It is clear from Table 1 that there is an increase in the value of revealed comparative advantage of Saudi dates from 39.4 in (1998-1999) to 61.2 in (2000-2002), and to 66.9 in (2003-2005).

Revealed Comparative Advantage for KSA and UAE's Dates Compared with the Most Important Competitor Countries. By comparing the revealed comparative advantage of KSA dates with the most important competing countries during the period (1998-2005), the results show that the reveled comparative advantage of KSA dates was about 55.8. This means that KSA dates are more competitive in the world market than Pakistan, the Emirate, Iran and Israel, where the revealed comparative advantages for dates of previous countries were 38.3, 21.9, 47.5 and 29.5, respectively as an average during the period (1990-2003) (Table 2). It is also clear that Saudi dates' revealed comparative advantage has continuously increased. It increased from 39.4 in 1998-1999 to 61.2 in 2000-2002 to 66.9 in 2003-2005. Table 2 shows the results of revealed comparative advantage for the most competing countries for KSA dates during 1998-2005. The results show that Saudi dates' revealed comparative advantage has continuously increased during the stated period, while that of UAE has fluctuated from 11 in 1998-1999 to 34 in 2000-2002.

There is an inefficient database, an unorganized marketing strategy and conflict between export companies with absence of cooperation (Elsabea, 2005). By studying manufactured dates the results show that about 90% of manufacturing activities concentrate on packaging (El-eid, 2008).

2. Competitiveness of Price Indicator. This paper aims at studying the export price per ton for the most important competing countries for Saudi dates as an indicator for the efficiency of the marketing system for these countries. The paper assumed that the difference of production and marketing cost between competing countries are insignificant and the main differences are prices of export. The results show that Israel dates had the highest export price by about $ 4,196 per ton, Tunisian dates had an export price of $ 1913, Algerian dates had a price of $ 1573. The KSA, Pakistan, Iran, the UAE and Iraq's dates follow by average export prices of $ 670, 379, 303, 212, 211 per ton, respectively, during the period 1998-2005.

By studying the ANOVA analysis between dates export prices for the most important export countries, the results were statistical significant at 5%. Calculated F was about 350.9 (Table 3).

By studying the LSD test the results shows that it is possible to divide countries according to their export prices into three groups.

The first group includes countries with the highest export prices such as Israel with an average price of $ 4,196 per ton. The second group includes countries with middle prices such as Tunisia, Algeria with an average export price of $ 1,743 per ton. The third group includes countries such as the KSA, Pakistan, Iran, the Emirates and Iraq with the lower prices by an average for the group of about $ 355.

It is clear from Table 4 that the KSA has a comparative price advantage for exporting dates compared with Israel, Tunisia and Algeria, where the price ratio for them is higher than one for the three periods of the study. On the other hand, Pakistan, Iraq, Iran and the UAE price ratios are less than one, which means that these countries have a comparative price advantage compared with KSA dates in the world market during all periods of study.

3. Market Share Indicator of KSA and UAE's Dates Compared with the Most Important Competing Countries. According to the agricultural ministry of the KSA the market share of Saudi dates in Europe is about 0.23 in Germany, 0.03 in France and zero in Italy, Spain and Switzerland in 2002 (Agricultural Research and Development Affairs, 2006).

The results of the study show that the exported quantity of KSA dates is concentrated in limited Arab countries (Table 5). About 49% of Saudi dates are exported to Yemen and about 81% of the total export is concentrated in six countries (Yemen, Kuwait, the UAE, Jordan, Syrian, and Qatar) with an average of 15675, 3031, 2879, 1602, 1596 and 1294 ton and representing 49.2, 9.5, 9, 5, 5 and 4.1% of the total KSA export (31,881 ton), respectively, during 2000-2002.

On the other hand, the exported quantity of UAE dates is concentrated in limited Asian countries (Table 6). About 32% of Emirates dates are exported to India and about 88% of the total export is concentrated in six countries (India, Bangladesh, Syrian, Srilanka, Indonesia and Pakistan) with an average 5941, 3694, 2088, 1034, 873, and 828 ton and representing 32, 20, 11, 6, 5 and 4% of the total UAE export (18,790 ton), respectively, during 2000-2002. These countries are considered as developing countries with low prices compared with developed countries. The exported quantity of KSA and UAE to Europe and America is very limited. So, it is important to pay more attention to these markets in the future through adapting a new production and marketing policy for producing suitable products to meet foreign consumers needs.

From Table 7, it is clear that the KSA has a market share about of 70% on the Syrian market. This means that 70% of Syrian import of dates comes from the KSA, then Qatar with 65%, followed by Yemen 53%, Kuwait 36%, Jordan 33%, Bahrain 25%, and Lebanon, Turkey, the UAE, Bangladesh, and Britain with 16, 5, 3, 2 and 2% during the period 2000-2002. The UAE play an important role for exporting and re-exporting dates especially for Asian countries but it is important to pay more attention to European countries and America by activating a complementary strategy with the KSA

4. Penetration Rate

Penetration Rate of KSA and UAE's Dates. The results show a strong penetration rate of

KSA dates for most Arab countries, especially Yemen, Syrian, Jordan, Kuwait and Lebanon during the period 2000-2002 (Table 8). This explains the ability of KSA dates to enter Arab markets. On the other hand, the KSA has a weak penetration rate for Asian and European markets. This means that it is difficult for the KSA dates to enter Asian and European markets. The highest value of Saudi dates penetration rate during the period 2000-2002 is about 32% in the Yemen market, then the Syrian market by about 31.6%, the Jordan market by 28%, Kuwait by 23.5%, Lebanon by 16%, Qatar by 7.8%, the Turkish market by 2.5%, followed by Bangladesh, the United kingdom, Bahrain, the Emirates by 2.1, 2, 1.1 and 0.4%, respectively. It is clear that KSA penetration of dates in the Emirates' market is weak. This insures the real situation as the Emirates are considered the fourth producer in the world. Export of dates from both countries needs to be organized to remove the negative impact on the production and marketing of dates and dates products. In reality, farmers of dates straggle from low prices in the KSA and the UAE because of the low level of marketing efficiency. By studying the penetration rate of dates for the UAE the results show that it was weaker than the penetration rate of KSA dates. Therefore, it is important for KSA and UAE dates to improve its competitive abilities in Asia and Europe and organize their ability to open new markets.

Comparing Market Share, Penetration Rate and Price Ratio of KSA and UAE Dates with the Most Important Competing Countries in the World Market. A range of authors have suggested that factors influencing the competitive advantage of organizations are two categories of determinants; (1) industry-level factors and (2) firm-level attributes (Rumelt, 1991; Lado et al., 1992; Amit and Schoemaker, 1993; Dyer and Singh, 1998, Lloyd, 2001).

By studying the competitive advantage of the KSA and the UAE the results show that the KSA has the first place in the Kuwait market for market share, penetration rate (Table 9). The most important competing countries for the KSA date are the UAE, Iran, and Tunisia (Agricultural Research and Development Affairs, 2006). It is clear that the KSA has a price advantage compared to Tunisian dates, but it does not have a price advantage over the UAE or Iran dates. In reality the manufacturing sector of dates needs to be more active to create new dates products. Therefore, it is important to build cooperation-marketing companies to promote the export of dates in both the KSA and the UAE and build a new marketing and manufacturing strategy for dates in both countries. Moreover, to remove all forms of negative effect between the two countries for dates and dates products.

For the Qatar market, the KSA comes in the first place for market share and second place after Iran for penetration rate. It is clear also that the KSA has a price advantage compared to the UAE and Iran but does not have a price advantage compared to Tunisia. The KSA comes in the first place for market share and penetration rate in the Lebanon market. In addition, the KSA has a price advantage compared to the UAE and Iran, but does not have a price advantage compared to Tunisia. The KSA comes in the second place for market share in the Bangladesh market after Pakistan and comes in the third place for penetration rate after the UAE and Pakistan. The price of KSA dates is higher than the UAE and Bangladesh.

The KSA has a weak competitive ability in the UK, where the KSA comes in the fifth place for market share and penetration rate after Iran, Tunisia, Israel and Pakistan. The most important competing countries in the UK market for KSA dates are Israel and Tunisia. This indicates that the KSA and the UAE have to improve their production and marketing abilities to meet foreign consumer's needs in Asia and Europe.

In the Bahrain market KSA dates have the first place for market share and penetration rate. The most important competitors are Tunisia and Iran, where the KSA has a price advantage for Tunisia but does not have a price advantage over Iran.

In the UAE Market, KSA dates come in the second place after Iran dates and followed by Pakistan as the UAE has re-exporting activities.

In the Turkish market, the KSA has the second place for market share and penetration rate after Iran. In reality, the KSA and the UAE have the abilities to increase

their dates exporting to Islamic countries in addition to Asian and European countries by meeting consumer's needs in these markets.

Although the KSA does not have a price advantage in the Yemen market, the KSA has the first place for market share and penetration rate and the UAE has the second place followed by Pakistan. Therefore, the KSA has a strong competitive situation in the Yemen market compared to other competitors and has about 53% of the Yemen market.

In the Jordan market, the KSA has the first place for market share and penetration rate. The UAE are the most important competitors in this market.

The results of the case study from KSA farmers, traders and manufacturers insure the previous results of secondary data. The results of primary data of the KSA, which include 150 farms, 35 traders in the whole market and 20 export companies selected randomly, insure the results of previous analysis of secondary data.

It shows that Arab export dates represented in both KSA and UAE's dates have difficulty to enter the European market because:

- European consumers prefer fruits without multi-color, without a high level of sugar.
- Most of the Arab dates production are dried kinds.
- European consumers prefer fresh fruits.
- Fresh Arab Rotab fail to be in a good situation after days during the export process.
- European consumers refuse fresh Arab Rotab with any color changes as they consider color changes a sign of fruit deterioration.
- Producing and marketing Arab bio-organic dates needs more attention.
- The strong competition between the KSA, the UAE Iran, and Pakistan's dates.
- Opening new markets present high costs.
- The exported quantity of high quality dates is small and cannot open new markets with high costs alone.
- Some exports of KSA and UAE dates suffer from infection with insect diseases.
- Arab products of dates suffer from unorganized production and marketing system.
- Importance of obtaining ISO (International Organization for Standardization), EUREP GAP and USDA Organic.
- Unavailable detailed database of production and marketing including importing countries, potential chances.
- Importance of building a production and marketing strategy for dates to open new markets in the future.

CONCLUSIONS

Arab dates represented in KSA and UAE's dates have to co-operate in production and marketing strategy in the light of reducing the negative impact of obstacle reasons during the production and marketing process of Arab export dates. It is important to produce according to consumers' needs in foreign countries, especially in Europe, America and Asia. Although the KSA and the UAE have strong competitive abilities in Arab countries, they have a weak competition in developed countries especially Europe and Asia. Moreover, the KSA and the UAE have a negative impact for producers of dates in both countries, which decreases the prices of dates and dates products in local and Arab markets.

Therefore, it is important to pay more attention to production and marketing co-operation between the KSA and the UAE for improving local and international markets of dates and remove negative impact on both countries of unorganized competition. The most important point of this paper is the importance of building a suitable strategy for both countries for dates to open new markets in the future, especially in Islamic countries, Asian, American and European countries according to consumers' needs of fresh, dried semi dried or manufactured Arab dates products. Finally, it is important to build cooperation-marketing companies to promote the export of dates in both the KSA and the UAE in addition to building a new marketing and manufacturing strategy for dates in both countries. This strategy has to depend on local and international information for

production, marketing and manufacturing of dates in addition to depending on quality and environmental standardization measures to improve competitiveness of Arabic dates in the future.

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Tables

Table 1. Revealed comparative advantage of Saudi and Emirat's dates during 1990-2003.

Period	Average value of world agri. export (1000 US $)	Average value of Saudi agri. export (1000 US $)	Average value of world dates export (1000 US$)	Average value of Saudi dates export (1000 US$)	RCA of KSA	RCA of UAE
1998-199	341712532	519624.7	200487.9	12016	39.4	11
2000-2002	360827775	529741.8	227465.9	20420.3	61.2	34
2003-2005	512629694	663976.9	341482.4	29593.7	66.9	20.8

Source: www.faostat.org, 2008.

Table 2. Average of dates revealed comparative advantage for the most important competitive countries for Saudi and Emirates' dates during the period 1998-2005.

Countries	Average period 1998-1999	Average period 2000-2002	Average period 2003-2005	General average
K.S.A	39.4	61.2	66.9	55.8
Pakistan	42.1	43.7	29.2	38.3
U.A.E	11	34	20.8	21.9
Iran	41.6	49	52	47.5
Israel	17	18.5	53.1	29.5

Source: www.faostat.org. 2008.

Table 3. Anova analysis for dates export price of the most important export countries in the world during the period 1998-2005.

Source of variation	*SS*	*df*	*MS*	*F*
Between countries	119985703	7	17140815	350.9
Inside countries	3126555.11	64	48852.42	
total	123112258	71		
LSD is significant at level 0.05				

Source: calculated from Table (3) in index.

Table 4. Competitive price indicator for KSA and UAE's dates compared with the most important competing countries during the period 1998-2005.

Period	Price of Israeli dates compared to Saudi dates	Price of Algerian dates compared to Saudi dates	Price of Tunisian dates compared to Saudi dates	Price of Pakistani dates compared to Saudi dates	Price of Emirates' dates compared to Saudi dates	Price of Iranian dates compared to Saudi dates	Price of Iraqi dates compared to Saudi dates
1998-1999	6	2.34	3.05	0.64	0.28	0.32	0.26
2000-2002	6	2.14	2.52	0.55	0.27	0.37	0.41
2003-2005	7	2.54	3.04	0.53	0.39	0.63	0.26
Average	6.20	2.34	2.87	0.57	0.31	0.44	0.31

Source:www.faostat.org, 2008.

Table 5. Share of Saudi dates export for the most important import countries during the period 2000-2002.

Countries	Price ($)	Quantity (ton)	Value ($ 1000)	% KSA dates export	Rank
Yemen	458	15675	7177	49.2	1
Kuwait	983	3031	2979	9.5	2
UAE	803	2879	2313	9.0	3
Jordan	615	1602	985	5.0	4
Syrian	359	1596	573	5.0	5
Qatar	750	1294	971	4.1	6
Lebanon	475	785	373	2.5	7
Turkey	340	456	155	1.4	8
Bangladesh	803	401	322	1.3	9
U. K	1181	232	274	0.7	10
Bahrain	793	184	146	0.6	11
Total		29367.0	16939.0	88.3	

Source:www.faostat.org, 2008.

Table 6. Share of Emirates' dates export for the most important import countries during the period 2000-2002.

Countries	Quantity (ton)	Value ($1000)	% of total export	Rank
India	5941	801	32	1
Bangladesh	3694	1089	20	2
Syrian Arab Republic	2088	374	11	3
Sri Lanka	1034	228	6	4
Indonesia	873	203	5	5
Pakistan	828	176	4	6
Morocco	714	125	4	7
Saudi Arabia	678	171	4	8
Yemen	526	131	3	9
Total quantity	16376	3298	88	

Source: www.faostat.org, 2008.

Table 7. Market share indicator of Saudi dates export during the period 2000-2002.

Countries	Average of total import of dates per ton	Imported quantity of KSA per ton	Market share
Syrian	2282	1596	70
Qatar	1990	1294	65
Yemen	17764	15675	53
Kuwait	1909	3031	36
Jordan	4921	1602	33
Bahrain	749	184	25
Lebanon	4967	785	16
Turkey	9470	456	5
UAE	196873	2879	3
Bangladesh	19477	401	2
UK	12009	232	2

Source: www.faostat.org, 2008.

Table 8. Penetration rate of KSA dates for the most important countries during 2000-2002.

Countries	Penetration rate
Yemen	32.1
Syrian	31.6
Jordan	28
Kuwait	23.5
Lebanon	16
Qatar	7.8
Turkey	2.5
Bangladesh	2.1
United Kingdom	2
Bahrain	1.1
UAE	0.4

Source:www.faostat.org, 2008.

Table 9. Dates market share, penetration rate and price ratio for the most important competing countries of the KSA and the UAE dates during 2000-2002.

Import market	Export countries	Market share	Price ratio	Penetration rate
Kuwait	1- KSA	36		23.5
	2- UAE	0.5	0.27	0.08
	3- Iran	2.5	0.37	2.52
	4- Tunisia	1	2.52	0.15
Qatar	1- KSA	65		7.8
	2- UAE	2	0.27	0.22
	3- Iran	13	0.37	12.96
	4- Tunisia	1	2.52	0.11
Lebanon	1- KS.A	16		16
	2- UAE	2	0.27	2.04
	3- Iran	4.8	0.37	4.81
	4- Tunisia	0.1	2.52	0.08
Bangladesh	1- KSA	2		2.1
	2- UAE	0.2	0.27	19
	3- Iran	0.6	0.37	0.6
	4- Pakistan	3.6	0.55	3.56
Britain	1- KSA	2		2
	2- UAE	0.002	0.27	0.15
	3- Iran	42.7	0.37	42.71
	4- Pakistan	3.3	0.55	3.43
	5- Tunisia	12	2.52	12.6
	6- Israel	5.8	6	5.91
Bahrain	1- KSA	25		1.1
	2- Iran	0.4	0.37	0.4
	3- Tunisia	3.3	2.52	0.15
UAE	1- KSA	3		0.4
	2- Iran	21.7	0.37	21.73
	3- Pakistan	0.2	0.55	0.06
Turkey	1- KSA	5		2.5
	2- Iran	68.1	0.37	68.06
	3- Tunisia	3.7	2.52	2
Yemen	1- KSA	53		32.1
	2- UAE	3	0.27	1.8
	3- Pakistan	0.4	0.55	0.14
Jordan	1- KSA	33		28
	2- UAE	7	0.27	6

Source: www. COM-trade.org, 2008.

Appendix

Table 1. Area of dates in Gulf countries by hectare during the period 1988-2007.

Years	KSA	UAE	Bahrain	Kuwait	Qatar	Oman
1988	64073	22156	1600	250	899	25000
1989	68305	22156	1600	400	967	25000
1990	72379	22156	997	350	998	25000
1991	75757	22368	950	250	1298	26000
1992	79575	27926	900	250	1537	27000
1993	83703	28860	920	500	1628	28000
1994	85790	28860	870	700	1700	28500
1995	93825	30215	812	750	1824	29000
1996	100858	31005	823	670	1897	29500
1997	106137	36531	823	890	2567	30000
1998	106460	59179	825	870	1368	35500
1999	141750	170330	830	1050	1366	35500
2000	142450	185330	823	1350	1931	35508
2001	139099	185330	823	1350	1516	33919
2002	139979	185329	1670	1350	1463	33869
2003	141421	185330	1670	1350	1464	33848
2004	148801	186000	1650	1450	1600	35532
2005	150744	185000	1700	1400	1444	31352
2006	151000	186000	1700	1400	1450	31352
2007	151000	186000	1750	1450	1450	31500

Source: www.FAO-Statistics Database, 2008.

Table 2. Production of dates in Gulf countries by ton during the period 1988-2007.

years	K.S.A	UAE	Bahrain	Kuwait	Qatar	Oman
1988	513613	141463	15000	1150	5270	120000
1989	521840	141463	15000	1954	5279	121000
1990	527881	141463	5712	1400	5712	120000
1991	528074	173110	6800	700	9013	135000
1992	552493	230400	8000	950	9521	150000
1993	563008	236135	9500	2100	10723	163000
1994	567762	236100	12000	3790	11431	170000
1995	589261	236965	16371	4410	12533	173000
1996	616908	244644	16508	5034	14582	180000
1997	649239	288190	16508	6662	22915	185000
1998	648000	290448	16600	6484	16409	126000
1999	712000	535964	16774	7894	16389	282000
2000	734844	757601	16508	10155	16116	280030
2001	817887	757601	16508	10376	13109	298006
2002	829540	757601	14500	12577	14845	238611
2003	884088	757601	14000	15811	16579	219770
2004	941293	760000	14000	16000	18222	231000
2005	970488	750000	15000	15800	19844	247331
2006	970000	755000	15000	14200	20000	258738
2007	970000	755000	15500	14500	21000	260000

Source: www.FAO-Statistics Database, 2008.

Table 3. Price of dates for the most important exporter countries during 1998-2005 ($/ton).

Years	Price of Iraq	Price of KSA	Price of UAE	Price of Algeria	Price of Tunisia	Price of Pakistan	Price of Israel	Price of Iran
1998	200	765	231	1836	2251	457	3193	221
1999	167	643	164	1460	2042	444	4583	226
2000	200	649	175	1368	1719	377	4556	240
2001	350	586	176	1330	1561	335	4509	239
2002	250	715	176	1482	1638	366	3568	243
2003	198	704	180	1612	1993	357	3922	289
2004	187	667	222	1790	2087	343	4756	385
2005	137	635	370	1702	2008	354	4480	585
Average	211	670	212	1573	1913	379	4196	303

Source: www.FAO-Statistics Database, 2008.

Table 4. Import and export quantity of dates for the most important import countries and their import from the KSA in the world during 2000-2002.

Countries	Exported quantity	Total production	Import from KSA	Total import
Yemen	262	31264	15675	17764
Kuwait	21	11036	3031	1909
UAE	288931	757601	2879	196873
Jordan	811	1614	1602	4921
Syrian	41	2808	1596	2282
Qatar	3	14690	1294	1990
Lebanon	63	0	785	4967
Turkey	779	9300	456	9470
Bangladesh	0	0	401	19477
Britain	288	0	232	12009
Bahrain	2	15839	184	749

Source: www.FAO-Statistics Database, 2008.

Figures

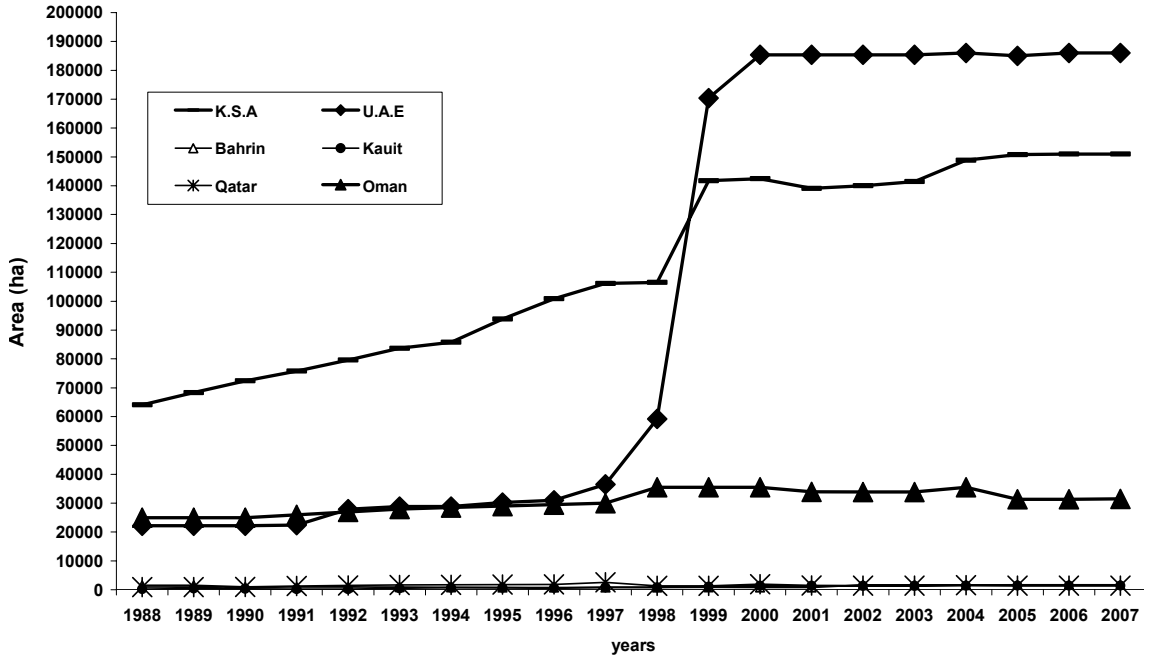

Fig. 1. Area of dates in Gulf region.

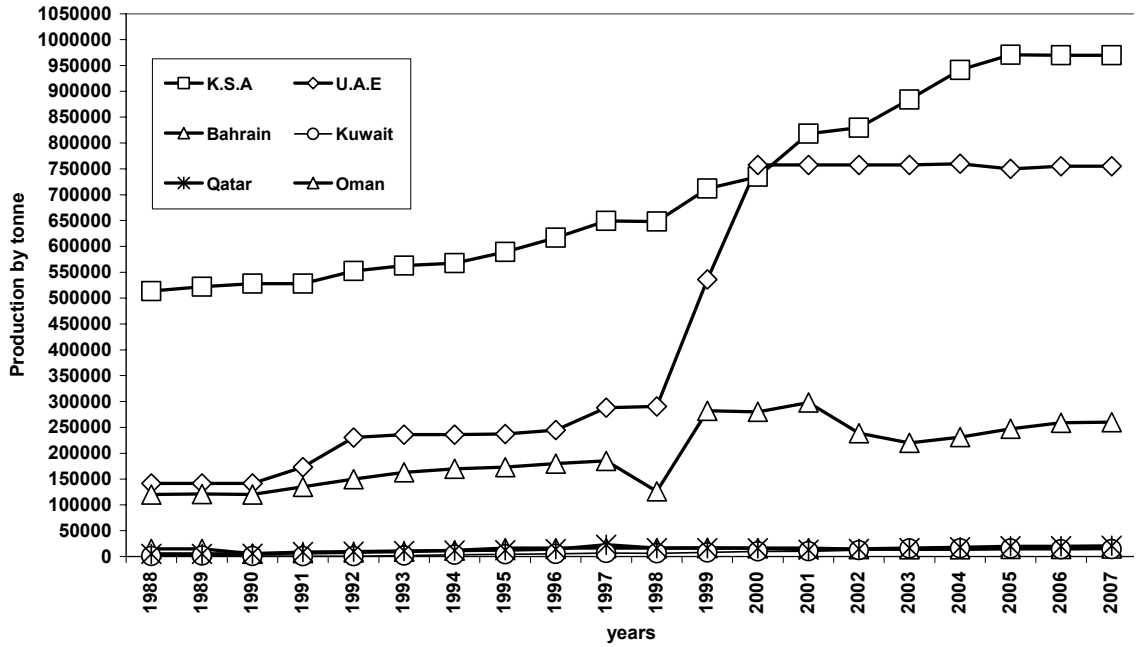

Fig. 2. Production of dates in Gulf region.

Safety Methods for Chlorpyrifos Removal from Date Fruits

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Abstract

The effectiveness of running water (H_2O), hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$), citric acid (CA) and acetic acid (AA) at concentrations of 1 and 2% for different dipping times was determined as postharvest washes for removing chlorpyrifos form date fruits treated at a concentration of 2 ppm. The recovered amount of chlorpyrifos was extracted based on the solid phase extraction (SPE) and then analyzed by gas chromatography with mass spectrometry (GC-MS). Results demonstrate that the removing of chlorpyrifos increased in the order of AA>CA>H_2O_2>$KMnO_4$>H_2O only. Also, the results showed that the percent of pesticide residue on date fruits depended on the concentration of tested washing treatments and dipping time. The formation of toxic by-product (chlorpyrifos oxon) was studied using gas chromatography-mass spectroscopy (GC-MS) analysis technique. No toxic by-product was identified in the extract of date fruits. Kinetic studies revealed that chlorpyrifos was found to be more easily removable from date fruits treated with the tested chemical solutions with $t_{1/2}$ values of 12-29 min compared with roughly 53 min in case of running water. The use of these washing treatments especially AA for removing chlorpyrifos as postharvest treatment may be an effective method. Studies are in progress to determine the impact of these washing treatments on the quality of date fruits.

INTRODUCTION

Pesticides are widely used in agriculture to control a variety of pernicious organisms that spoil the crops. More than 600 kinds of agrochemicals are used around the world (Miyake et al., 1999). They provide unquestionable benefits for agricultural production, even though, as a consequence, low amounts of some residues may persist in the food supply and could constitute a significant exposure pathway for humans. Exposure to food residues has created uncertainty for potential chronic toxicity and in some cases, acute toxicity (Saunders and Harper, 1994; Ekström et al., 1996; Osman and Al-Rehiayani, 2003).

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad spectrum organophosphorus (OP) insecticide, widely used in agriculture and public health, found in trace levels in vegetables (Unpublished data) and possesses low water solubility (1.40 mg/L) and a high octanol-water partition coefficient (K_{ow}=4.70). It acts as non-systemic insecticide with contact, stomach, and respiratory action and is used to control *coleoptera*, *diptera*, *homoptera* and *lepidoptera* in soil or on foliage in over 100 crops, including pome fruit, stone fruit, citrus fruit, bananas, vines, vegetables, etc. (Tomlin, 2002). Despite recent restrictions on further production for use, chlorpyrifos remains the most widely used organophosphate pesticide, and there is increasing concern over the potential consequences of fetal and childhood exposures (Song et al., 1998). The acute toxicity of chlorpyrifos is mediated through inhibition of cholinesterase by the active metabolite chlorpyrifos oxon, and the consequent accumulation of the neurotransmitteracetylcholine (ACh) in synaptic junctions leads to excessive stimulation

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of postsynaptic cells causing cholinergic toxicity (Ecobichon, 1996), but new evidence suggests that chlorpyrifos itself may influence DNA synthesis, brain cell replication and differentiation directly (Crumpton et al., 2000; Dam et al., 2000). Also, chlorpyrifos has been postulated to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress thereby disrupting normal cellular development and differentiation (Osman, 1999; Bebe and Panemangalore, 2003).

A number of physical-chemical and conventional methods, such as Fenton oxidation (Wang and Lemley, 2002), biotreatment (Liu et al., 2004), TiO_2 catalytic treatment (Kouloumbos et al., 2003), powdered activated carbon filtration and reverse osmosis have been demonstrated to be highly effective for the removal of organic chemicals including pesticides (Heijman and Hopman, 1999). Those techniques mainly focused on pesticides dissolved in aqueous solutions. However, they are less effective or unsuitable for removal of residual pesticides adhering on vegetable surface. Products currently used as food additives or for sanitary purposes could be selected for washings. Citric acid is authorized as food additive in the EU (E330 antioxidant) and used as an acidulant and synergistic antioxidant in pharmaceutical preparations. The use of hydrogen peroxide (H_2O_2) for the treatment of both drinking-water and wastewater has increased in recent years. H_2O_2 is often added as an oxidant, or it is used for the control of surplus chlorine contents or for the removal of residual ozone contents in processed waters. H_2O_2 is a very common disinfectant included in the US Pharmacopoeia (at 3% (v/v)). Potassium permanganate ($KMnO_4$) is an antiseptic and disinfectant that can be employed for drinking water treatment at 0.01% (w/v); additionally, it reacts with ethylene delaying the maturation of fruits. In addition, all these compounds are environmentally friendly because they are all natural substances and/or can be easily degraded in the environment.

Date palms (*Phoenix dactylifera* L.) are a staple food in the diet of many countries and are considered as the major fruit of the Near East and North Africa where they are consumed in large quantities fresh, dried, or in various processed forms since they are rich in carbohydrate (mainly glucose, fructose and a small amount of sucrose), protein and minerals (Considine, 1982; Al-Showiman and Fayadh, 1990). In the Kingdom of Saudi Arabia (KSA), dates are one of the most important crops because of their religious and nutritional significance. Saudi Arabia is the largest date producer in the world, with a production amounting to 970,488 tons tones per year and the number of date palm trees is over 18 million (Anon, 2004). Al-Qassim region is one of the largest agricultural areas in the KSA where much of its arable and fertile land is under date palm orchards. Al-Qassim Community produces the majority of date fruits consumed locally and much of those are exported outside the country. During the 2002 season, date fruits production was greater than 130,000 tons, of which approximately 1,092 tons were destined for export (Saudi Ministry of Agriculture and Water, 2003). As a result of its high economic value as well as the large number of pests that infest dates during growth, significant quantities of pesticides are often necessary for the protection of this crop. A number of pesticides are registered by the Ministry of Agriculture in Saudi Arabia to control these pests, which infest dates and cause severe damage (Al-Rehiayani and Osman, 2003, 2005). Chlorpyrifos is widely applied to date palm in the KSA at rate of 250 ml/100 L to control fruit worms, caterpillars, mites and aphids. This leads to pesticide residues on (or in) the fruits at harvest. Unfortunately, no data are available on chlorpyrifos removing from date fruits, although it has been reported in fruits and vegetables (Krol et al., 2000), nectarine (Pugliese et al., 2004), rice grains (Kaushik et al., 2009), and water (Sherrard et al., 2004).

The search for means to improve the production of dates in the KSA is always the target of scientists, politicians and businessmen, who seek new techniques to enhance the quality and safety of this product. Therefore, the present study was carried out to evaluate the effectiveness of running water (H_2O), hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$), citric acid (CA) and acetic acid (AA) at concentrations of 1 and 2% for different contact times as simple wash treatments for reducing chlorpyrifos residues from date fruits and the possibility of toxic by-products formation by these

treatments was investigated by gas chromatography-mass spectroscopy (GC-MS).

MATERIALS AND METHODS

Chemicals

Analytical grade standard for chlorpyrifos, (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), was obtained from Chemservice, USA, with 99% purity, while formulated chlorpyrifos (48% a.i., EC) was purchased from the local market of Al-Qassim region. Certified HPLC-grade of actone, methanol, cyclohexane and ethyl acetate were purchased from BDH Company, while the Water spe-20G Column Processor designed vacuum manifold capable of processing up to 20 solid phase extraction (SPE) columns and SPE columns (Waters spe^{TM}, C18, 500 mg per column) were purchased from Waters, USA. Acetic acid (100%), citric acid (trisodium salt dehydrate), hydrogen peroxide (30%) and potassium permanganate (>99%) were obtained from BDH, Bio-Rad Lab., WinLab and Sigma Companies, respectively. Ultra-pure deionized water of 15 MΩ cm resistivity was obtained from a water purification system (PURELAB Option-R, ELGA, UK) and used throughout this study. All other chemicals used in this study were of the highest grade available.

Date Fruits Treatment

The date fruits (*Phoenix dactylifera* L.) used in this experiment were the 'Succary' variety and were obtained from organic farming without the use of pesticides provided by a rural cooperative located in Al-Qassim region, KSA. They were harvested in September 2009 and were untreated post-harvesting. The 0.042 ml of formulated chlorpyrifos was mixed with 10 L ultra-pure deionized water to give a concentration of 2 ppm. Fresh and unblemished pesticide-free fruits were immersed into chlorpyrifos solution for 2 min with gentle rotation by hand. Date samples with pesticide on the surface were then air-dried for about 24h under room conditions.

Removal of Residual Pesticide from Date Fruits

Removal of chlorpyrifos on date fruits was studied at concentrations of 1 and 2% of either acetic acid (AA), citric acid (CA), hydrogen peroxide (H_2O_2) or potassium permanganate ($KMnO_4$), and four different contact times (5, 15, 30 and 60 min) at room temperature (25±1°C). Triplicate random date samples spiked with chlorpyrifos were divided into the following treatment groups: control (no wash); rinsing in running tap water; AA, CA, H_2O_2 and $KMnO_4$.

Sample Preparation and Solid-Phase Extraction

Samples were chopped and a subsample (10 g) was weighed into 50 ml Teflon centrifuge tube and extracted with 20 ml acetone using a homogenizer (Euroturaax, IKA Labortechnik Staufen, Germany) at full speed for 2 min. The extract was centrifuged at 3000 rpm for 5 min and the supernatant was transferred to a clean graduated cylinder (25 ml) to measure its volume. Solid-phase extraction was carried out using SPE columns preconditioned by passing 6 ml of ethyl acetate followed by 6 ml of methanol and then 8 ml of ultra-pure deionized water according to the Štanbaher and Zupančič-Kralj method (2003). The extract was quantitatively transferred to a 2 ml clean vials and completed with ethyl acetate/cyclohexane (1:1) to 1 ml.

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography (Model GC 450, Varian Inc., The Netherlands) with a mass spectrometry (MS 220.41) detector equipped with split/splitless injector with electronic pressure control was employed. A Fused silica CP-Sil 8 CB-LB/MS capillary column (30×0.25 mm i.d.) was used in combination with the following oven temperature programme: initial temperature 50°C, 5°C/min ramp to 290°C held for 10 min. The injector temperature was 280°C. The carrier gas (helium, 99.999%) flow rate was set to a

constant head pressure of 200 kPa at flow rate of 1.0 ml min^{-1} with split ratio of 1:20 min. The mass spectrometer was operated in electron ionization mode with a transfer line temperature of 280°C, manifold temperature 40°C, ion trap temperature 200°C, filament number 2, ion source 240°C and selected ion monitoring (SIM) mode. The ion energy for electron impact (EI) was kept at 70 eV. MS Workstation version 6.9.1. was used for data acquisition. For positive identification, both retention time (Rt) and the presence of five fragment ions (*z/m* ions: 352, 314, 258, 197 and 97) were considered.

Calibration Curves

Stock solution of chlorpyrifos (1000 µg/ml) was prepared in acetone and then serial dilutions ranging from 0.01 to 10 ng/µl were prepared using ethyl acetate/cyclohexane (1:1, V/V). Areas under the peak versus concentrations were plotted and fit by simple linear regression to obtain the equation for the standard curves for the tested pesticides. The amount of chlorpyrifos in fruits was thus calculated based on the slope of the standard curve.

Recovery Studies

Recovery tests were carried out by using pesticide-free sample at 0.05, 0.10 or 0.50 mg/kg of chlorpyrifos and then extracted as described previously. The recovery values ranged from 81 to 115% which are considered acceptable (Herdman et al., 1988), while precision ranged from 2 to 12%. Limits of detection (LOD) and quantitation (LOQ) ranged from 0.002 to 0.010 and 0.006 to 0.020 mg/kg, respectively

Statistical Analysis

Data were calculated as mean ± SD analyzed using ANOVA. A probability of 0.05 or less was considered significant. The statistical package of the Costat Program (1986) was used for all chemometric calculations.

RESULTS AND DISCUSSION

Removing of Chlorpyrifos by Different Wash Treatments

Washing is generally the first step in various types of treatments which are given to food commodities in combination (like washing followed by cooking, washing and drying, washing and peeling and washing, peeling and juicing) to allow for effective decontamination from pesticides. The solutions for washing fruits must be of low toxicity and easily biodegradable in order to allow their use at home and at processing-food industries. Postharvest treatments, such as the postharvest water wash and scrub that have been traditionally employed to remove debris and dirt from apples to reduce pesticide residues (El-Hadidi, 1993). The use of postharvest chlorine dips has also shown potential as an effective postharvest treatment in the reduction of pesticide residues on apple fruits (Hendrix, 1991).

In the present study, the effects of some home preparations on chlorpyrifos residues in the date fruits were investigated by using gas chromatography-mass spectrometry. These methods include washing by either running tap water and two levels (1 and 2%) of either AA, CA, H_2O_2 or $KMnO_4$. With this study, we hope to understand how home treatments influence pesticide residues in date fruits and to guide citizens on how to remove pesticide residues effectively. The amount of chlorpyrifos was significantly decreased exponentially as the contact time increased in the fruits treated with different wash treatments (Fig. 1). There were significant variations between all the tested treatment and control (chlorpyrifos only) with respect to their abilities to remove chlorpyrifos within the contact times. Also, there were significant variations between all treatments when the contact time was 5 min and the remaining contact times.

Data presented in Table 1 show the chlorpyrifos concentrations in date fruits after spiking and the residual pesticide concentrations after different wash treatments. Among these washing methods, the washing by running tap water proved the least effective,

showing 37.08, 42.46, 51.77 and 51.93% loss when the contact times were 5, 15, 30 and 60 min, respectively, compared with no wash treatment (control). The present results are in parallel with that found by Abou-Arab (1999) who reported that washing tomatoes by tap water proved the least effective, showing 9.62, 15.3, 9.17, 18.8, 22.7 and 16.2% loss of HCB, lindane, p,p-DDT, dimethoate, profenofos and pirimiphos-methyl, respectively. Also, the initial diazinon residue level (0.822 ppm) on cucumbers was decreased by 22.3% by washing for 15 s by rubbing under running water (Cengiz et al., 2006). On the other hand, washing rice grains with water removed approximately 60% of the chlorpyrifos residues (Lee et al., 1991). In addition, rinsing fruits and vegetables with tap water for 15-30 s produced significant reductions in residue levels of malathion, iprodione and other pesticides but not of chlorpyrifos (Krol et al., 2000).

In the present study, rinsing at 1% AA removed 81.12, 88.91, 91.93 and 98.42% of residual pesticide, while rinsing at higher concentration of acetic acid (2.0 %) increased the efficiency in pesticide removal where the percentages of chlorpyrifis removing were 87.23, 93.36, 99.17 and 99.5% when the contact times were 5, 15, 30 and 60 min, respectively. In case of CA, washing with 1% CA solution caused 72.46, 78.85, 90.43 and 92.33% loss in chlorpyrifos, while at the higher concentration (2%), the percentages of chlorpyrifos removing were 77.81, 84.12, 92.39 and 95.84% when the contact times were 5, 15, 30 and 60 min, respectively. Also, the results showed that when date fruits contaminated with 2 ppm of chlorpyrifos and then washed with 1% H_2O_2 solution the percentages of chlorpyrifos removal were 47.33, 73.79, 76.30 and 77.80%, while at a concentration of 2% the percentages of chlorpyrifis removal were 59.82, 79.55, 81.56 and 84.13% when the contact times were 5, 15, 30 and 60 min, respectively. In the case of date fruits treated with chlorpyrifos and then washed with 1% $KMnO_4$, roughly 64% of the available chlorpyrifos was removed within the first five min, a percentage that increased to about 70-75% within 15-60 min. In the case of date fruits treated with 2% $KMnO_4$, 69-73% of the available chlorpyrifos was removed within the first 15 min; this increased to about 84% within 30-60 min. These findings are in accordance with those reported by Zhang et al. (2007) who found that washing with AA solutions (at 10% concentration for 20 min) caused 79.8, 65.8, 74.0 and 75.0% loss of chlorpyrifos, *p,p*-DDT, cypermethrin, chlorothalonil from cabbage, while washing by tap water (for 20 min) caused 17.6, 17.1, 19.1 and 15.2% loss. On the other hand, washing nectarines treated with chlorpyrifos, fenarimol, iprodione, malathion, methidathion, myclobutanil, parathion and pirimicarb with aqueous CA, H_2O_2, $KMnO_4$, sodium hypochlorite, sodium metabisulfite, and urea solutions produced results without significant differences with those obtained with the tap water (Pugliese et al., 2004). Thus, the main mechanism for removing the residues is dissolution and chemical degradation is unappreciable. From the results obtained in this research work and assuming the criterion that a treatment is efficient in degrading pesticides if a removal percentage of above 70% is obtained (Ormad et al., 2008). The present study revealed that removing of chlorpyrifos from date fruits depends on the levels of tested chemical solutions and the contact times which are in accordance with that by Abou-Arab (1999) who reported that the loss of HCB, lindane, p,p-DDT, dimethoate, profenofos and pirimiphos-methyl depends on the levels of acetic acid and NaCl solutions. The use of such simple and non-toxic washing treatments to reduce such residues in fruit samples (Krol et al. 2000). Also, the amount of pesticide removed by washings is related to its water solubility and the octanol-water partition coefficient (Pugliese et al., 2004).

One of the health concerns of using oxidants to degrade pesticide is the formation of toxic intermediates. The present study investigated the efficacy of two oxidizing agents ($KMnO_4$ and H_2O_2) to remove chlorpyrifos from date fruits. $KMnO_4$ and H_2O_2 were assayed for washings having redox properties, and thus, can modify the chemical structure of the selected pesticides creating a derived by-product that is more toxic than the parent pesticide. If it is the case, such washing treatment should not be utilized to reduce the pesticide residue levels in fruits. It is well known that some organophosphorus pesticides containing P=S bonds (actually organothiophosphorus pesticides) such as

chlorpyrifos react with oxidative reagents producing its respective oxygen analogs (e.g., chlorpyrifos-oxon), which are more potent as mammalian acetylcholinesterase inhibitors than the parent forms (Amdur et al., 1991). The possible formation of toxic by-products by either $KMnO_4$ or H_2O_2 that are oxidative reagents was investigated by gas chromatography-massspectrometry (GC-MS) in SCAN mode by monitoring *m*/*z* ions: 109, 197, 242, 270, and 298 for chlorpyrifos oxon. Only single peak at a retention time of 12.14 min corresponding to chlorpyrifos was observed in the GC-MS chromatogram and there is no intermediate or dead-end product detected using the analytical method described in the present study (Fig. 2). No toxic by-products (chlorpyrifos-oxon, malaoxon, methidaoxon and methyl paraoxon) were identified in the extracts of the washed samples for the washing-times and concentrations studied, but at high levels of sodium hypochlorite, $KMnO_4$ and H_2O_2 formed oxons from the organophosphorus pesticides (Pugliese et al., 2004). There are many factors that affect the activity of the oxidative reagents to form oxons such as solvent, pH, identity of the pesticide, levels of reagents, the reagent itself, reaction time, temperature, endogenous matrix compounds (Pugliese et al., 2004). Advanced oxidation processes (AOPs) are the hydroxyl radical-mediated oxidations which utilize hydroxyl radicals ($^{\bullet}OH$) as their primary oxidizing species and have proven to be very effective in treating a wide variety of organic contaminants leading not only to their destruction, but also, given sufficient conditions, to their complete mineralization with no more toxic compound that can be produced during the degradation process (Glaze et al., 1987; Buxton et al., 1988; Benitez et al, 2002). Furthermore, the radicals produced from these oxidants can substitute halogens attached to aromatic rings, thus generating biodegradable compounds, although the reaction takes place in a slow rate (Haag and Yao, 1992). Products of chlorpyrifos degradation include 3,5,6-trichloro-2-pyridinol which subsequently breaks down to organochlorine compounds and carbon dioxide (The Royal Society of Chemistry, 1988). H_2O_2 enhanced the photodegradation of parathion through the reaction between UV generated hydroxyl radical and parathion yielding several organic byproducts, of which the paraoxon, 4-nitrophenol, O,O,O-triethyl thiophosphate and O,O-diethyl-methyl thiophosphate (Wu and Linden, 2008). A higher concentration of H_2O_2 results in a higher steady-state hydroxyl radical concentration and thus increases the availability of hydroxyl radical to degrade parathion (Wu and Linden, 2008). Also, H_2O_2 acts as a hydroxyl radical scavenger producing a much less reactive $^{\bullet}HO_2$ radical. This scavenging effect becomes significant at higher hydrogen peroxide concentrations and thus less hydroxyl radical is available for degrading parathion.

Kinetic Studies

A biphasic model was assumed in order to carry out the statistical study of the loss of chlorpyrifos according to equation (1).

$$C_t = A_0 e^{-\alpha t} + B_0 e^{-\beta t} \quad (1)$$

where, C_t is the recovered amount of chlorpyrifos at t min, A_0 and B_0 are the concentrations of chlorpyrifos at zero time, while, α and β are the disappearance rate constants for the first and second and phases, respectively. The half-life ($t_{1/2}$) of the exponential decay was calculated according to equation (2).

$$t_{1/2} = (2.303 \log 2)/\text{rate constant} \quad (2)$$

The data fitting results in case of different wash treatment using second order kinetic showed that coefficients of determination (R^2) ranged from 0.988 to 0.999 (Table 2). The biphasic model is characterized by a rapid phase (first phase), and a much slower phase (second phase). This is clearly reflected in the $t_{1/2}$ values, where the half-lives of chlorpyrifos were 53.33, 13.86, 12.60, 21.01, 19.25, 12.60, 12.16, 28.88 and 27.73 min, while, the half-life values ($t_{1/2}$) values for chlorpryrifos in the second phase model were

138.65, 30.14, 17.33, 33.01, 46.22, 15.07, 21.01, 57.77 and 53.33 min when date fruits were contaminated with chlorpryrifos at a concentration of 2 ppm and treated with running water, 1% AA, 2% AA, 1% CA, 2% CA, 1% H_2O_2, 2% H_2O_2, 1% $KMnO_4$ and 2% $KMnO_4$, respectively (Table 2). The fate of chlorpyrifos in aquatic systems has been especially well-studied, with reported half-lives of 4.7 to 7 hours (Schaefer and Dupras, 1970; Knuth and Heinis, 1992). Also, The rate of reaction of chlorpyrifos (vide supra) with $HO^{\bullet}$ in the gas phase at high temperatures was studies with half-life of 1.4h (Hebert et al., 2000).

The present findings are in accordance with those of many investigators who reported that the kinetics of pesticide degradation is commonly biphasic with a very rapid degradation rate at the beginning followed by a very slow prolonged dissipation (Alexander, 1994; Jones et al., 1996; Rigas et al., 2007; Osman et al., 2009). The relative importance of the phases depends on the availability of the pollutants, hydrophobicity, and affinity for organic matter. On the contrary, first order removal rate for of $0.039h^{-1}$ was recorded for chlorpyrifos simulated stormwater runoff treated in constructed wetland mesocosms (Sherrard et al., 2004).

CONCLUSIONS

Washing with water and various chemical solutions for domestic use are necessary to decrease the intake of pesticide residues. Since the wash treatment dosage and treatment time could impact the efficiency in pesticide removal, the better removal efficiency could be obtained through optimizing these parameters. Chlorpyrifos was significantly removed more from date fruits in all the tested wash treatments at all the tested time intervals compared with unwashed-fruits (treated with chlopyrifos only). By the end of the experiment almost complete chlorpyrifos removing occurred in AA-washed fruits. Citric acid washing came next in importance to washing by acetic acid solutions, giving remove 92-96% of the compound removing at the end of the experiment. By contrast, contaminated fruits treated with H_2O_2 and $KMnO_4$ gave 78-84 and 75-84% of the compound removing at the end of the experiment, respectively. Although, tap water wash treatment significantly reduced the tested pesticide residual levels on date fruits compared with no wash treatment, but still less effective than the other treatments. The removing pattern could be attributed to the low water solubility and high octanol-water partition coefficient of chlorpyrifos. Also, the present study revealed that there was a gradual increase in the percent of reduction due to the increase of concentrations of AA, CA, H_2O_2 or $KMnO_4$ for same-contact time treatment as well as extension of contact time could increase the efficiency in pesticide removing.

Kinetic studies revealed that chlorpyrifos was found to be more easily removable from date fruits treated with the tested chemical solutions with $t_{1/2}$ values of 12-29 min compared with roughly 53 min in case of running water. By the end of the experiment almost complete chlorpyrifos removing occurred when date fruits were washed with acetic acid followed by citric acid and then hydrogen peroxide and potassium permanganate. The rate of chlorpyrifos removing by chemical solutions was almost 1.85-4.39 times faster than running water.

In Saudi Arabia, and other neighboring countries, natives consume more date fruits (more than 10 times) than an average person living outside this region. Such high date consumption could lead to a higher risk of exposure to pesticides, especially in children and other vulnerable individuals because date fruits are freshly consumed, therefore, consumers are increasingly concerned about chlorpyrifos residues in date fruits and their carry-over to processed products. Therefore, the present study validated that water and/or acetic acid, citric acid, hydrogen peroxide or potassium solutions as wash treatments are safe and promising processes for the removal of the chlorpyrifos from date fruits surface under domestic conditions. Results found in the present study must not be extrapolated to other pesticides, crops or conditions.

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Tables

Table 1. Effect of different wash treatments on removing of chlorpyrifos from date fruits.

Wash treatment	Contact time (min)							
	5		15		30		60	
	Chlorpyrifos levels (µg/kg)	Remove (%)	Chlorpyrifos levels (µg/kg)	Remove (%)	Chlorpyrifos levels (µg/kg)	Remove (%)	Chlorpyrifos levels (µg/kg)	Remove (%)
Chlorpyrifos	32.90±2.20aH	-	32.81±4.55aF	-	32.70±3.11aF	-	32.20±4.87aG	-
Chlorpyrifos+ Running water	20.70±1.50bG	37.08	18.88±2.18aE	42.54	15.77±1.53aE	51.77	15.48±2.23aF	51.93
Chlorpyrifos+ 1% Acetic acid	6.21±0.63cB	81.12	3.64±0.21bB	88.91	2.64±0.25bB	91.93	0.51±0.10aAB	98.42
Chlorpyrifos+ 2% Acetic acid	4.20±0.21cA	87.23	2.18±0.27bA	93.36	0.27±0.11aA	99.17	0.16±0.05aA	99.50
Chlorpyrifos+ 1% Citric acid	9.06±0.76cC	72.46	6.94±0.22bC	78.85	3.13±0.27aB	90.43	2.47±0.34aC	92.33
Chlorpyrifos+ 2% Citric acid	7.30±0.99cB	77.81	5.21±0.85bB	84.12	2.49±0.44aB	92.39	1.34±0.35aBC	95.84
Chlorpyrifos+ 1% H_2O_2	17.33±2.530bF	47.33	8.60±1.05aD	73.79	7.75±0.68aD	76.30	7.15±1.23aE	77.80
Chlorpyrifos+ 2% H_2O_2	13.22±3.11bE	59.82	6.71±1.50aC	79.55	6.03±0.87aC	81.56	5.11±1.01aD	84.13
Chlorpyrifos+ 1% $KMnO_4$	11.99±1.21cE	63.56	9.73±1.06bD	70.34	8.37±1.22aD	74.40	8.00±1.02aE	75.16
Chlorpyrifos+ 2% $KMnO_4$	10.21±0.67cD	68.97	8.82±0.67bD	73.12	5.34±0.899aC	83.67	5.22±3.54aD	83.79

Each value is the mean ± S.D of three replicates with 6 determinations. Means having the same small letter in the same raw are not significant ($P<0.05$).
Means having the same capital letter in the same row are not significant ($P<0.05$).

Table 2. Kinetic parameters for the chlorpyrifos dissipation in date fruits in presence of different wash treatments.

Wash treatment	Kinetic parameters						
	A_0 (µg/kg)	B_0 (µg/kg)	α (min^{-1})	β (min^{-1})	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	Regression coefficient (R^2)
Chlorpyrifos + Running water	21.90	16.05	0.013	0.005	53.33	138.65	0.988
Chlorpyrifos+1% Acetic acid	7.60	4.60	0.050	0.023	13.86	30.14	0.999
Chlorpyrifos+2% Acetic acid	5.25	0.35	0.055	0.040	12.60	17.33	0.999
Chlorpyrifos+1% Citric acid	10.60	3.80	0.033	0.021	21.01	33.01	0.997
Chlorpyrifos+2% Citric acid	8.25	3.65	0.036	0.015	19.25	46.22	0.999
Chlorpyrifos+1% H_2O_2	21.05	9.05	0.055	0.046	12.60	15.07	0.999
Chlorpyrifos+2% H_2O_2	16.40	7.10	0.057	0.033	12.16	21.01	0.999
Chlorpyrifos+1% $KMnO_4$	13.10	8.85	0.024	0.012	28.88	57.77	0.999
Chlorpyrifos+2% $KMnO_4$	11.70	5.50	0.025	0.013	27.73	53.33	0.994

Each value is the mean of three replicates with 6 determinations. Means in the same row followed by the same letters are not significantly different ($P \leq 0.05$, Duncan's multiple-range test).

Figures

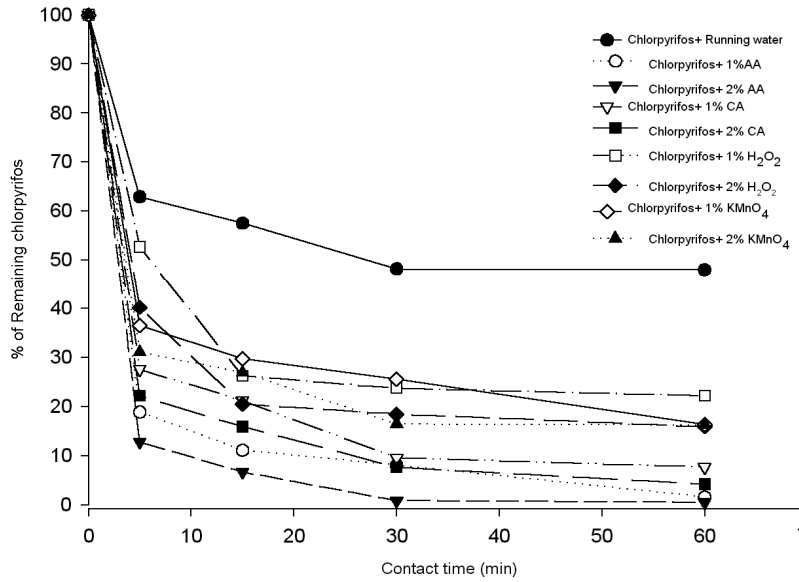

Fig. 1. Removing curves for chlorpyrifos from date fruits by different wash treatment.

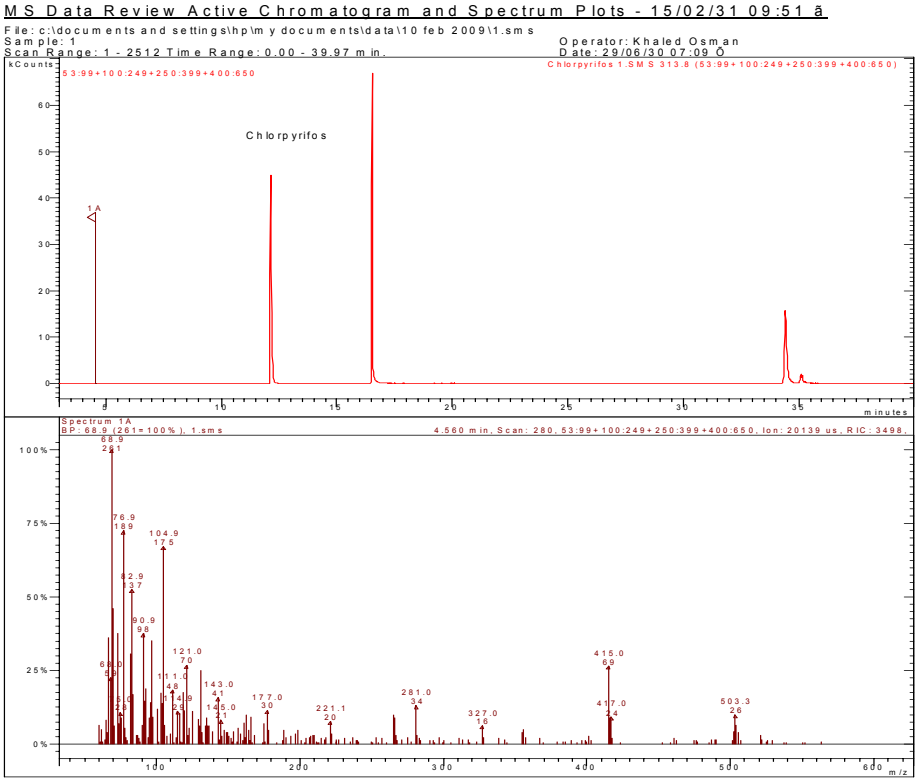

Fig. 2. GC-MS chromatogram corresponding to date fruits immersed in 2 ppm chlorpyrifos and washed by different treatment.

The Effect of Feeding Date Palm By-Products on Ewes and Lamb Intake and Performances

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Keywords: date palm by-product, sheep and lamb, intake, growth

Abstract

Two experiments were conducted on D'Man ewes. Date palm by-products (DPB) are whole dates rejected for human consumption. A total of 11 DB varieties were sampled and analyzed. In Exp. 1, suckling ewes (n=20) were used during 90 d to compare a control diet to a DPB diet. Whole or seedless DB nutritive composition, feed intake and lamb BW were measured. In Exp. 2, concentrate was progressively substituted by the DPB mixture during 21 d in 12 ewes. The initial daily offer of DPB was 0.4 kg and the final was 0.7 kg with an increase rate of 50 g every 3 d. The composition of DB changed according to the variety, the rangesbeing : CP (1.99 to 4.50%), NDF (10.52 to 26.05%) and ADF (7.15 to 21.96%). Whole DB have higher contents than seedless DB: CP (3.24 vs. 2.88%), NDF (18.11 vs. 13.93%), ADF (13.01 vs. 9.77%) and ADL (5.99 vs. 5.63%), with a 5% net energy difference (1.92 vs. 2.02 Mcal NEL/kg DM, *P*=0.071). In Exp. 1, lambs ADG between 10 and 45 d, did not vary according to feeding treatment (C, 113+22; DB, 128+13 g/d). In Exp. 2, ewes feed intake decreased as the rate of concentrate substitution by DB increased. Total daily intake decreased from 1.64 to 1.31 kg from the beginning to the end of the experiment, as a consequence of the decrease of hay intake (0.24 kg/d) and refusing of DB seeds (0.093 kg/d). In conclusion, DPB can substitute concentrate at a minimum rate of 25% in sheep diets, although fill value increases with DB incorporation.

INTRODUCTION

In recent years, the price of classic raw materials for animal feeds has increased dramatically. Incorporation of by-products can be one way to reduce the animal feeding costs. The date palm production has a high importance in Tunisia. This activity is represented by 4,200,000 of palm trees on 35,000 ha. Average production of date is about 140,000 to 160,000 t among which 60,000 t is intended for export. Livestock are a very important activity in oases, mainly sheep and goats. Animal feeds in oases areas are largely imported from the north, in particular the grass hay or straw, the barley and the industrial concentrate feeds. Date palms production generates an appreciable amount of by-products that can be used in the animal feeding, principally discarded dates, date pits, dry leaves and peduncles (Genin et al., 2004). Date supplemented diets may improve animal performance and could be used as an energy source to replace a part of the concentrates in the ration. The present study was carried out to evaluate nutritional characteristics of some date palm by-products, and to study the effect of replacing a part of dietary concentrate by low quality dates on intake and growth of ewes and lambs.

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MATERIAL AND METHODS

DPB Evaluation

11 samples representing the dominant of low quality date palm varieties are collected from Tozeur region in the western South of Tunisia. Samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), and total ash by standard procedures (AOAC, 1975). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the Goering and van Soest (1970) method, using an automated fibre analyser.

Animal Performances

Two experiments were conducted on D'Man ewes in the station of Degueche situated in the western South of Tunisia. In Exp. 1, twenty suckling ewes were used during 90 d to compare a control diet (C) composed by 1.0 kg oats hay and 0.7 kg concentrate, as fed, to a date palm by product (DPB) diet which contains 1 kg oats hay, 0.35 kg concentrate and 0.35 kg DPB mixture. Feed intake and lamb body weight were measured. In Exp. 2, concentrate was progressively substituted by the DB mixture during 21 day in 12 ewes. Initial daily offer of DPB was 0.4 kg and final was 0.7 kg with an increase rate of 50 g every 3 days. In all experiments, hay was fed once daily and concentrate or DPB were fed twice daily in equal portions AM and PM.

Parameters of chemical composition, pH, dry matter intake (DMI), and average daily gain (ADG) were studied with descriptive analysis and one way ANOVA with general linear models procedure, according to the Snedecor and Cochran (1984) method and using the Minitab software. The dietary treatment effect was tested according to the following model:

$$Y_{ij} = \mu + P_i + e_{ij} \quad (1)$$

where, μ = mean; P_i = effect of dietary; e_{ij} = the experimental error.

RESULTS

Results of chemical analyses of the whole and seedless samples of the 11 varieties of dates are shown in Table 1.

Composition of date palm by-products varied among varieties (Table 1), the ranges being: CP (1.99 to 4.50%), NDF (10.52 to 26.05%) and ADF (7.15 to 21.96%). In general, these figures are in agreement with those reported by El-Hag et al. (1993), Genin et al. (2004) and Sawaya et al. (1983). Whole 'Guendi' varieties have the highest crude protein and fiber levels. Whole DPB have higher contents than seedless DPB in CP (3.24 vs. 2.88%), NDF (18.11 vs. 13.93%), ADF (13.01 vs. 9.77%) and ADL (5.99 vs. 5.63%), with a 5% lower net energy (1.92 vs. 2.02 Mcal NEL/kg DM, P=0.071).

Results about animal intake and performances of Exp. 1, show that DPB mixture composition was intermediate comparatively to the previous samples (3.04% CP, 15.24% NDF, 11.05% ADF). There were no significant differences in average daily gain (ADG) between 10 and 45 day for the two diets in D'men lambs. Lambs ADG was respectively equal to 113±22 and 128±13 g for the control and experimental group. Lambs in the control group consumed a same quantity of feed expressed as total feed intake as lambs fed DPB supplemented diets.

In Exp. 2, ewes the feed intake decreased as the rate of concentrate substitution by DPB increased (Fig. 1). Total daily intake decreased from 1.64 to 1.31 kg from the beginning to the end of the experiment, as a consequence of the decrease of hay intake (0.24 kg/d) and refusing of DPB seeds which are estimated to 0.093 kg/d. High temperature conditions at the last week of the experiment can also explain the decreases in ewes intake.

DISCUSSION

Addition of discarded dates to ruminant diets does not affect the productivity of lambs and ewes. On the basis of the chemical composition, date-palm by-products are relatively comparable to alfalfa hay with the exception of their CP content, and can be compared with medium quality cereals with low level in nitrogen (Chehma and Snoussi, 2010). As reported by other authors (Boudechiche et al., 2008), there are great and significant differences between date varieties in fiber and protein content, but these differences are reduced in mixtures of dates. Net energy is 5% higher in seed less dates due to lower level of NDF, ADF and ADL. It was known that high fibre feeds result in lower energy value, and previous research has demonstrated the contribution of cell wall to the depression in ration intake (Keady and Mayne, 2001). The lower crude protein in seedless dates does not agree with results of Sawaya et al. (2006) who reported a level of 6.5% of CP in seeds. This effect can be related to cultivar differences.

Under similar fattening conditions and at the same age, lambs fed a classic diet or a DPB content diet have equal average daily gains. The moderate level of DPB in the experimental diet explains the comparable levels of growth levels of lambs. The total consumption evolves regularly with progressive substitution of energetic concentrate by the same quantity of DPB and protein correction of the diet. Environmental conditions affected total feed intake by reducing hay intake. Replacing 50% of concentrate with a same amount of DPB did not change feed intake significantly, which may be due to the high palatability of rations containing DPB (Abdel-Rahman et al., 2002).

CONCLUSIONS

DPB are an interesting alternative for animal feeding in the oasian system, it can substitute hay, straw and a part of concentrate in sheep and lamb diets. A diet containing DPB must be balanced for nitrogen composition to maintain the level of intake and animal performances.

ACKNOWLEDGMENTS

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Tables

Table 1. Chemical analyses of the whole and seedless samples of the 11 varieties of dates.

Varieties	DM (%)	Ash (%)	OM (%)	CP (%)	E.E.	NDF (%)	ADF (%)	ADL (%).	Net Energy (Mcal/kg DM)
Alig W	89.26	2.43	97.57	3.91	1.76	26.05	19.26	7.57	1.74
Alig S	92.75	2.66	97.34	2.78	1.64	25.39	16.90	5.02	1.81
Kentichi W	90.87	2.63	97.37	2.87	0.17	10.52	7.75	5.27	2.07
Kentichi S	92.57	2.91	97.09	2.65	0.32	14.74	9.31	5.18	2.03
Khwatt W	86.66	3.13	96.87	2.16	0.89	16.51	12.18	4.64	1.95
Khwatt S	86.68	3.38	96.62	1.99	0.21	10.60	7.93	5.10	2.07
Khalt W	86.72	2.34	97.66	3.81	0.95	18.66	11.84	4.02	1.96
Khalt S	86.67	2.08	97.92	3.51	0.25	11.50	7.15	3.56	2.09
Alig 2 W	88.90	2.57	97.43	2.86	0.74	16.68	12.58	7.19	1.93
Alig 2 S	86.75	2.70	97.30	3.28	0.18	14.21	10.42	8.21	2.00
Khalt K W	88.92	2.60	97.40	3.32	1.16	21.43	14.08	6.68	1.89
Khalt K S	89.43	2.61	97.39	3.24	0.10	15.79	11.92	6.78	1.95
Ftimi W	88.33	2.89	97.11	2.96	0.26	10.62	8.02	5.46	2.07
Ftimi S	89.70	2.62	97.38	2.63	0.15	10.21	7.67	5.05	2.08
Guendi W	89.75	3.73	96.27	4.50	2.12	30.61	21.96	7.77	1.66
Guendi S	85.36	3.95	96.05	3.80	0.29	12.85	9.44	6.23	2.03
Degla W	88.64	3.17	96.83	2.84	0.49	14.82	11.15	6.01	1.98
Degla S	91.17	2.46	97.54	2.20	0.18	11.91	9.79	4.56	2.07
Mean W	88.75	2.83	97.17	3.24	0.92	18.11	13.01	5.99	1.92
Mean S	88.95	2.77	97.23	2.88	0.35	13.93	9.77	5.63	2.02
Mean	88.85	2.80	97.20	3.06	0.64	16.02	11.39	5.81	1.97

W: Whole dates.
S: Seedless dates.

Figures

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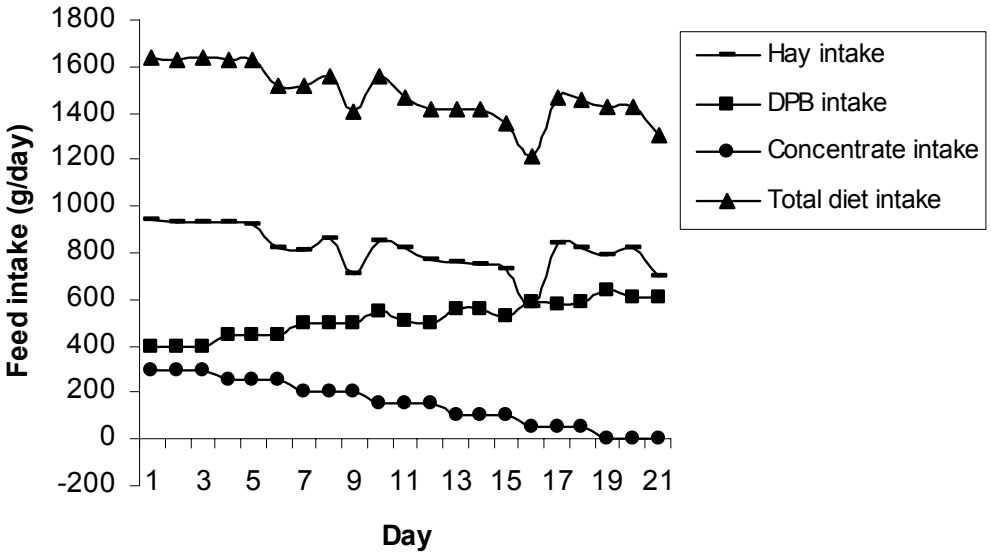

Fig. 1. Total intake by ewes of feed including DPB.

Antimicrobial Activity of Date Palm (*Phoenix dactylifera*) Pits Extracts and Its Role in Reducing the Side Effect of Methyl Prednisolone on Some Neurotransmitter Content in the Brain, Hormone Testosterone in Adulthood

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Keyword: pits of date palm (*Phoenix dactylifera*), antimicrobial, methyl prednisolone, *Klebsiella pneumonia*, *Escherichia coli*, neurotransmitter content

Abstract

The present work was carried out to study the impact of date palm pits' antibacterial activities on two species of pathogenic bacteria (*Klebsiella pneumonia* and *Escherichia coli*) and its role in reducing the side effect of methylprednisolone on some neurotransmitter content in the brain, hormone testosterone and testculture of male albino rats. Date palm pits are most effective in inhibiting growth of bacteria as compared with antibiotics due to differences in resistance of bacteria to anti-tested materials due to the change in membrane permeability of cells, thereby hindering the entry of enzymes or excreted by the change in the chemical composition of the constituent chemical. The results showed that the daily oral administration of pits of date palm caused the maximal increase in NE, DA and GABA content that was found in the brain stem after 2 weeks. The daily oral administration of methylprednisolone caused a decrease in NE, DA and GABA content found in the brain stem after 2 weeks. Moreover, the daily oral administration of pits of date palm and methylprednisolone caused an increase in NE content found in the brain stem after 2 weeks. The daily oral administration of pits of date palm and methylprednisolone caused a significant increase in testosterone level in serum blood of male albino rats. From the present results, it is clear that the effect of chronic oral administration of methylprednisolone and pits of data palm caused a recovery effect on testicles of male albino rats, noticing the high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules.

The appropriate recommendations in this study are to use nuclei dates antimicrobial on *Klebsiella pneumonia* and *Escherichia coli* than the activity of standard antibiotics and the results concluded that using intended date palm pits as a preventive measure to reduce the side effects resulting from the use of a drug methylprednisolone on the some neurotransmitter content in the brain. The hormone testosterone was determined in a testculture of male albino rats.

INTRODUCTION

The date palm (*Phornix dactylifera* L.) is considered the most important source of food for humans in both arid and semiarid regions (Besbes et al., 2004). Dates contain a high percentage of sugars reaching 88% in some varieties (Al Shahib and Marshall, 2003). Dates are also rich in mineral salts and vitamins (Booij et al., 1992). For the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose this is 1.68 and 1.53, respectively (Fayadh and Al-Showiman,1990). In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative (Zohget et al., 2000). Experimentally, date extracts have

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been shown to increase the sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (El-Mougy et al., 1991). Date palm pollen (DPP) cure mal-infertility by improving the quality of sperm parameters (Bahmanpour et al., 2006). Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone (Ali et al., 1999).

The pits of *Phoenix dactylifera* contain different chemical compounds such as saturated and unsaturated fatty acids, Zinc (Zn), Cadmium (Cd), Calcium (Ca) and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty acids contain linoleic and oleic acids which could inhibit the 5-α reeducates enzyme (Shariati et al., 2008). Also, dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall, 1993). Studies indicate that the aqueous extracts of dates have potent antioxidant activity (Mansouri et al., 2005). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (Gu et al., 2003; Al-Farsi et al., 2005).

In recent years, it has been suggested that estrogen may be involved in regulating the renewal of spermatogonial stem cells (Miura et al., 2003) and male reproductive tissues with estrogen receptors (Amin et al., 1969). Investigations have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Reports have also pointed that isolation of micro elements from DPP has estrogen, sterols, and other agents that may influence male fertility (Bennet et al., 1966; Mahran et al., 1976; Bajpayee et al., 1997). With regard to these components, snack foods have been supplemented with date pollen to improve male infertility (Abde-El-Mageed et al., 1987).

The present work was carried out to study the impact of date palm pits' antibacterial activities on two species of pathogenic bacteria (*Klebsiella pneumonia* and *Escherichia coli*) and its role in reducing the side effect of methylprednisolone on some neurotransmitter content in the brain, the level of the hormone testosterone and on a testculture of male albino rats.

MATERIALS AND METHODS

Materials

1. Date Palm (*Phoenix dactylifera* L.) Pits. It belongs to kingdom: *Plantae*, division: *Magnoliophyta*, class: *Liliopsida*, order: *Arecales*, family: *Arecaceae*, genus: *Phoenix*, species: *Phoenix dactylifera* and the binomial name: *Phoenix dactylifera* Linn. Date fruits were obtained from the Al-Gaseem Date Factory in the central region of the Kingdom of Saudi Arabia.

2. Drug. Methylprednisolone at a dose of 20 mg/kg through the infectious oral tube once daily (Park, 1998).

3. Bacterial Test. *Klebsiella pneumonia* and *Escherichia coli* from the laboratory of the Jeddah Hospital in the kingdom of Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37°C.

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines (Ryan and Ray, 2004). It is clinically the most important member of the *Klebsiella* genus of *Enterobacteriaceae*; *Klebsiella* was named after the German bacteriologist Edwin Klebs (183-1913).

Escherichia coli is a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). It is now classified as part of the *Enterobacteriaceae* family of gamma-proteobacteria (Thompson, 2007). Lee, et al. (2009) found that pathogenic *E. coli* found in meat in Korea could act as a transmission vehicle for human infection as suggested by the occurrence and classification of pathogenic *E. coli* in retail meats. Furthermore, the data from their study

could be used in the risk assessment of foodborne illnesses linked to meat consumption.
4. Animals. Adult Male albino rats, weighing (100 to 120 g), were obtained from the Experimental Animal House Center, King abdualaziz University, Jeddah, Saudi Arabia. All animals were given food (rat chow or date extract) and water ad libitum, and were maintained at a relative humidity of 65 to 86%, at a temperature of 23 to 25°C, and in a schedule of 12 hours of light and 12 hours of dark. Rats were weighed at the beginning and end of the study. Procedures involving animals and their care were conducted in conformity with international laws and policies.
5: Standard Antibiotic Disc. Amikacin, Gentamicin, Imipene, Cefotaxime, Cefe-pime, Aztreonam, Piperacillin, Amoxicillin-Clavulanate, Tazobactam, Colistin, Nitrofurantion, Ciprofloxacin, and Norfloxacin Mast Diagnostic Amiens, France.

Methods
1. Preparation of *Phoenix dactylifera* (Date Palm) Pits. The dried pits were ground into a fine powder and immersed in cold distilled water (1:3 ratio, weight to volume) for 48h at a temperature of 4°C. The water extract was prepared freshly and given to the animals ad libitum in place of rat chow (Al-Qarawi et al., 2004).
2. Antibacterial Activities. The agar disc diffusion method was employed for the determination of antibacterial activities of the water extract of *Phoenix dactylifera* pits powder (Hasenekoğlu, 1990). The suspension of the tested microorganisms (106 CFU/µl) was spread on Mueller Hinton Agar (Oxioid) for bacteria, filter paper discs (6 mm in diameter) were soaked with 20 µl of the stock solutions and placed on the inoculated plates. After keeping at 2°C for 2h, they were incubated at 37°C for 24h. The diameter of the inhibition zones were measured in millimeters. Some known antibiotics Amikacin, Gentamicin, Imipene, Meropenem, Cephalothin, Cefuroxime, Ceftazidime. Cefotaxime, Cefe-pime, Aztreonam, Ampicillin, Piperacillin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam, Colistin, Nitrofurantion, Ciprofloxacin, Norfloxacin and Tetracyclin were evaluated for their antibacterial activities and their results compared with *Phoenix dactylifera* pits extract (Baker and Breach, 1980).
3. Animal Treatment. The animals were divided into four groups (n=4) of equal number, three experimental and control groups. The control group only received an equal volume of distilled water. The control group 1 was orally and daily administered the equivalent amount of the vehicle (distilled water) for the same period. The second group was orally and daily administered methylprednisolone at a dose of 20 mg/kg and the third group was orally and daily administered pits of date palm (20 mg/kg b.wt.). Later, the third group was orally and daily administered 4 ml of pits of date extract (20 mg/kg b.wt.) for 15 consecutive days and treated with methylprednisolone (20mg/kg) with pits of data extract. At the end of the experimental periods (2 weeks), rats were scarified under diethyl ether anesthesia at fasting state.

During the treatments four rats of each group were decapitated each week till the end of the 2-week duration times. The rats were killed by sudden decapitation at the designed times. The brain was rapidly and carefully excised, and was then dissected according to the method of Glowinski and Lversen (1966) into the following regions; cerebellum, striatum cerebral cortex, hypothalamus, brain stem and hippocampus, brain content was wiped dry with filter paper, weighed, wrapped in plastic films and then in aluminum foil and were quickly frozen in dry ice, pending analysis. NE and DA were extracted and estimated in the brain tissues according to the method of Chang (1964) modified by Ciarlone (1978). GABA were extracted and estimated in the brain tissues according to the method of Sutton and Simmonds (1973).
4. Blood Sampling. Portions of blood samples were collected and allowed to coagulate at room temperature; EDTA (ethylene diamine tetracetic acid) was added to the other portion of blood and centrifuged at 3000 rpm for 30 min. The clear, non-haemolysed supernatant serum and plasma were quickly removed, divided into four portions for each individual, and stored at -20°C for subsequent analysis. For the measurement of testosterone the immunoassay technique and spectra were used. Testosterone kits were

used according to their manufacturer's instruction (Orion Diagnostica; Finland and DRG Instruments GmbH; Germany).

5. Histological Studies. After sacrificing the animals, part of the testculture from each animal from the treated and the control groups was removed and immersed in 10% buffered formalin solution, the test culture was kept in separate numbered small glass bottles. The test culture was then embedded in paraffin, and sectioned. Four sections (5 microns in thickness) were taken from each testculture, each section being at a distance of at least 500 ų from the proceeding one. Sections were stained with haematoxylin and eosin (Harris, 1900). The scoring system for tubular affection was determined (mean no. of dysfunctioning tubules per 5 fields ×100), 0-1 representing all tubules showing active spermatogenesis; 1-2 representing a portion of tubules showing arrest or hypospermatogenesis; 2-3 representing all tubules showing arrest or hypospermatogenesis, but germ cells are intact; 3 representing all tubules showing arrest or hypospermatogenesis, but germ cells are partially or completely replaced.

6. Statistical Analysis. Values reported are means ± SE (n=6). The results were statistically analyzed using the Student's t-test (Hill, 1971) for unpaired data, with P value of less than 0.05 considered significant.

RESULTS

The emergence of the inhibition zone of the growth of pathogenic bacteria *Klebsiella pneumonia* and *Escherichia coli* as a result of various transactions, with extract of pits of data palm is cleqr from Tqble 1. It was found that when the transaction intended to pits of data palm extract the reported inhibition zone formed around the filter papers was saturated with about 16.351±0.00 and 10.00±0.32 mm, respectively, and similar to the impact of anti-vital Cefotaxime with the impact of intended dates on the bacteria *K. pneumonia*. The inhibition zone of bacteria *E. coli* appeared to a lesser extent, reaching 4.33±21.0 mm while the adversaries Aztreonam and Amikacin offer results similar for each of the two genera. The antibiotic Colistin had less effect in the inhibition of bacteria tested, reaching 0.833±0.211 and 0.533±0.021 mm for each of the *K. pneumonia* and *E. coli*, respectively.

It is noticed that the difference in the sensitivity of bacteria through the difference in the inhibition zone around discs saturated and date palm pits is most effective in inhibiting growth of bacteria as compared with anti-vital due to differences in resistance of bacteria to anti-tested materials.

The presented results in Table 2 and Figure 2 show that the daily oral administration of pits of data palm (20 mg/kg b.wt.) resulted in a significant increase in DA content starting from the 1st week in the cerebellum, the cerebral cortex, the brain stem and the hippocampus and in the same tested areas from the 2nd week till the end of the experimental duration. The maximal ($p<0.01$) increase in DA content was found after 2 weeks in the cerebellum (+38.69%). Table 3 and Figure 3 show that the daily oral administration of pits of data palm caused a significant increase in GABA content standing from the 1st week in the cerebellum, the striatum, the cerebral cortex and the brain stem. From the 2nd week in all tested areas except the hypothalamus and the hippocampus. The maximal ($p<0.001$) increase in GABA content was found after 2 weeks in the brain stem (+324.35%).

As shown in Table 4 and Figure 4, the daily oral administration of pits of data palm (20 mg/kg b.wt.) caused a significant increase in NE content starting from the first week in the cerebellum, and from the second week in the cerebellum and the striatum and the maximal ($p<0.01$) increase in NE content was found in the cerebellum after 2 weeks (+41.22%).

The presented results in Table 5 and Figure 5 show that the daily oral administration of methylprednisolone (20 mg/kg b.wt.) resulted in a significant decrease in DA content starting from the 1st week in the whole brain area till the end of the experiment duration and in the same tested areas from the 2nd week till the end of the experimental duration. The maximal decrease ($p<0.001$) in DA content found after 2

weeks in the hypothalamus (-78.94%). Also, Table 6 and Figure 6 show that the daily oral administration of methylprednisolone caused a significant ($p<0.001$) decrease in GABA content starting from the 1st week in the whole brain area till the end of the experiment duration except the cerebral cortex and in the same tested areas from the 2nd week till the end of the experimental duration. The maximal decrease in GABA content found after 2 weeks in the hypothalamus (-77.48%).

The results presented in Table 7 and Figure 7 show that the daily oral administration of methylprednisolone (20 mg/kg b.wt.) caused a significant decrease in NE content starting from the 1st week in the whole brain area till the end of the experiment duration. The maximal decrease ($p<0.001$) in NE content was found in the hippocampus after 2 weeks (-82.05%).

As shown in Table 8 and Figure 8, the daily oral administration of pits of data palm and methylprednisolone resulted in a significant increase in DA content starting from the 1st week in the cerebral cortex and decreased in the cerebellum, the striatum, the brain stem, the hypothalamus and the hippocampus from the 2nd week and increased in the cerebellum, the striatum and the cerebral cortex. Decrease in DA content was found in the hypothalamus and the hippocampus. The maximal ($p<0.001$) increase in DA content was found after 2 weeks in the cerebral cortex (+312.86%), the maximal ($p<0.01$) decrease in DA content found after 2 weeks in the striatum (-37.19%). Table 9 and Figure 9 show that the daily oral administration of pits of data palm and methylprednisolone caused a significant increase in GABA content standing from the 1st week in the cerebral cortex. From the 2nd week this increased in the striatum and the cerebral cortex and decreased in all tested areas. The maximal ($p<0.001$) increase in GABA content was found after 2 weeks in the cerebral cortex (+72.84%). The maximal ($p<0.01$) decrease in GABA content was found after 1 week in the hippocampus (-54.68%). As shown in Table 10 and Figure 10, the daily oral administration of pits of data palm and methylprednisolone caused a significant increase in NE content starting from the 1st week in the cerebellum, and from the 2nd week in the cerebellum and the striatum and the maximal ($p<0.01$) increase in NE content was found in the cerebellum after 2 weeks (+41.22%).

The results presented in Table 10 and Figures 11, 12 and 13 show that the daily oral administration of methylprednisolone caused a significant decrease in testosterone level in serum blood of the male albino rat. The maximal ($p<0.05$) decrease in testosterone level was found after 2 weeks (-22.89), while the daily oral administration of data palm pits and date palm pits with methylprednisolone caused a significant increase in testosterone level in serum blood of male albino rats. The maximal ($p<0.001$) increase in testosterone level was found in after 2 weeks (218.46 and 72.37, respectively).

The presented results show (Plates 1a and b) histologically, small normal seminiferous tubules mostly without lumen, surrounded by a fibrous connective tissue layer with high magnification showing dark spermatogonium and pachytene spermatocytes and Sertoli cells. Also note the myoid cell layer surrounded and the intertubular space containing leydig cells. Plates 1c and d show enlarged normal seminiferous tubules populated by spermatocytes and late spermatids surrounding the tubular lumen and high power showing light and dark spermatogonium adjacent to the basal lamina; late spermatids with elongated head directed towards Sertoli cells.

The normal testicular architecture, interstitial cells and tubules show active spermatogenesis with normal central luminal mature sperms. Tubular dysfunction (i.e., hypospermatogensis and germ cell maturation arrest) are within the normal range. No organic pathological lesions (i.e., absent interstitial fibrosis, congestion, vascular injury or inflammation with no tubular necro-degenerative changes or atrophy).

The results presented in Plate 2 show the effect of chronic oral administration of pits of date palm on testicles of male albino rats causing the tubules of testicles showing an increased active spermatogenesis with a significant rise in the number of mature sperms. No interstitial fibrosis, congestion, vascular injury or inflammation with no tubular necro-degenerative changes or atrophy were found, as shown in Plate 2c, there

was a marked increase in spermatogenesis, free of early or late arrest.

The results presented in Plate 3 show the effect of chronic oral administration of methylprednisolone on testicles of male albino rat causing a marked reduction of spermatogenesis (hypo-spermatogenesis) and tubules showing partial late arrest with a marked reduction of mature sperms. As shown in Plate 3c, there was an early arrest with absent mature sperms and germ cell hypoplasia. As noticed in Plate 3d, there are focal areas of disrupted architecture and the tubules show absent spermatogenesis (mostly early arrest). Also, in Plate 3e, foci of interstitial fibrosis, congestion, vascular injury 'endarteritis' and inflammation are also encountered with tubular necro-degenerative changes, as well as atrophy of both germ and interstitial cells.

From the presented results, it is clear that the effect of chronic oral administration of methylprednisolone and pits of date palm on testicles of male albino rats caused a recovery effect. Notice the high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules. There is minimal interstitial fibrosis but no vascular injury 'end-artritis obliterans', or tubular necro-degenerative changes or atrophy (Plate 4).

The results show that the daily oral administration of pits of date palm caused a significant decrease in tubular dysfunction and arrest in testicles of male albino rats. The daily oral administration of methylprednisolone caused an increase in tubular dysfunction and an arrest in testicles. Moreover, the daily oral administration of pits of date palm and methylprednisolone (recovery) caused reduced tubular dysfunction and testicular arrest (Fig. 14).

DISCUSSION

Date palm pits is most effective in inhibiting growth of bacteria as compared with antibiotics due to differences in resistance of bacteria to anti-tested materials due to change in membrane permeability of cells, thereby hindering the entry of enzymes or excretions by the change in the chemical composition of the constituent chemical or by changing the nature of some of their components targeted by the anti-abstract (Aba Al-Khail et al., 2003). These results agree with Jassim et al. (2007) wo found that the date pit extracts show a strong ability to inhibit the infectivity of *Pseudomonas* phage ATCC 14209-B1 and completely prevented bacterial lysis. This effect was shown to be due to interference with some aspect of the phage's lytic cycle. The lytic cycle of the phage consists of three major phases (Stewart et al., 1998): binding to a suitable host bacterium and injection of its genome; a period of intracellular production of new virions; and then lysis of the cell and release of progeny phage into the environment. This effect was shown to be due to a direct effect of the extract on the phage itself rather than an effect on the host cell. This finding is supporting by Mansouri et al. (2005) who indicated that the aqueous extracts of dates have potent antioxidant activity. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (Gu et al., 2003; Al-Farsi et al., 2005).

From the present results, it is clear that the daily oral administration of pits of date palm caused a reducing side effect of Methylprednisolone on some neurotransmitter content in the brain and a significant increase in neurotransmitter contents (NE, DA and GABA) in most of the tested brain areas at different time intervals; the cerebellum which is responsible for the voluntary movement; pons+medulla oblongata which is responsible for essential reflexive acts; the striatum which is a brain region responsible for motor activity; the cerebral cortex is responsible for sensation including visual, auditory and olfactory as well as motor coordination and association, also it responsible for higher mental functions such as thinking, planning, reasoning, memory and consciousness and the hippocampus. This is key area concerned with learning (Bloom, 2001). The brain stem is responsible for integration of coordination of essential reflexive acts such as swallowing, vomiting and respiration (Bloom., 2001).

This is in agreement with the previous studies which suggested that the methanolic extract of *P. dactylifera* possesses significant anxiolytic, analgesic, nootropic and

antipsychotic activities which may be attributed to various mechanisms such as decreased serotonergic and dopaminergic transmission and increased cholinergic transmission. These findings scientifically validated the traditional claim and suggested its valuable role in the treatment of various CNS disorders (Vyawahare et al., 2009). Various parts of *P. dactylifera* are widely used in traditional medicine for the treatment of various disorders, which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders, etc. (Nadkarni,1976). Date fruit extracts have been reported to possess antiulcer, anticancer, antidiarrheal, hepatoprotective, antimutagenic, antioxidant, aphrodiasiac, antiinflamatory, antimicrobial, antigenotoxic, antihyperlipidemic and nephroprotective activities (Vayalil et al., 2002; Doha et al., 2004; Al-Qarawi et al., 2005; Ishurda et al., 2005; Allaith et al., 2005; Bahmanpour et al., 2006; Jassim and Naji., 2007; Abdulla and Al Taher, 2008; Mohamed et al., 2008). Additionally, the PD extract did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the retard model, suggesting that the inhibitory effect of the extract might be elicited via central mechanisms, not by peripheral neuromuscular blockade, and also ruled out the possibility of neurotoxicity (Dunham and Miya, 1967; Amos et al., 2001). Abdullah et al. (2004) have suggested that the reduction of CCl4-induced elevated plasma activities of AST, ALT, ALP, and the bilirubin level in animals pre- and post-treated with the aqueous extracts of date flesh or pits shows their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent CCl4 hepatotoxicity.

The daily oral administration of pits of date palm and methylprednisolone caused a significant increase in the testosterone level in serum blood of male albino rat. This result agrees with Kostyuk (2004) who indicated that a date palm pollen suspension increases the plasma levels of estradiol and testosterone and these hormones are found at high concentrations in rat testis and seminal fluids. Also, Zargari (1999) found that date extracts increase the sperm count in guinea pigs and increase the concentration of testosterone, follicle stimulating hormone, and latinizing hormone in rats. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone (Nayernia, 2004).

From the present results, it is clear that the effect of chronic oral administration of methylprednisolone and pits of data palm on testicles of male albino rats caused a recovery effect. Notice the high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules. There is minimal interstitial fibrosis, but no vascular injury ‘end-arthritis obliterans’, or tubular necro-degenerative changes or atrophy. This finding is supported by Bahmanpour et al. (2006) who found that the comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis. It did not significantly affect the weight of the prostate and the seminal vesicle or the histology of the reproductive content. Zargari et al. (1999) have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Our data showed that using pits of data palm increases the plasma levels of testosterone. This hormone is found at high concentrations in testis and seminal fluids of rats (Kostyuk et al., 2004). Mahran et al. (1976) indicated that date palm contains estradiol and flavonoid components that have positive effects on the sperm quality.

CONCLUSIONS

As a conclusion, the appropriate recommendations in this study are to use nuclei dates antimicrobial properties on *Klebsiella pneumonia* and *Escherichia coli* than the activity of standard antibiotics and the results concluded that using intended date palm pits as a preventive measure to reduce the side effects resulting from the use of the drug methylprednisolone on some neurotransmitter content in the brain, hormone testosterone in male albino rats. From the presented results, it is clear that the effect of chronic oral

administration of methylprednisolone and pits of date palm on testicles of male albino rats caused a recovery effect, noticing the high sperm in some tubules. These results confirm that pits of date palm had beneficial effects on male reproductive activity and improve sperm quality and enhance fertility in the male adult rat. Therefore, it may be useful to solve infertility problems.

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Tables

Table 1. Diameter of inhibition zone of the date palm (*Phoenix Dactylifera* L.) Pit againt *Klebsiella pneumonia ssp pneumonia* and *Escherichia coli*.

	Treatment					
	Date palm (*Phoenix dactylifera* L.) pit	Amikacin	Gentamicin	Imipene	Cefotaxime	Cefepime
	Inhibition zone (mm)					
Klebsiella pneumonia	16.351±0.00	13.333±1.687	6.00±1.265	1.00±000	16.33±0.211	16.50±0.224
Escherichia coli	10.00±.032	14.00±0.730	7.00 ±0.632	1.00± 0.00	4.33±0.211	15.00±0.305
	Treatment					
	Aztreonam	Amoxicillin Clavulanate	Colistin	Nitrofurantion	Ciprofloxacin	Norfloxacin
	Inhibition zone (mm)					
Klebsiella pneumonia	14.667±0.422	8.667±0.422	0.833±0.211	15.667±0.279	1.167±0.279	1.917±0.083
Escherichia coli	15.33±0.422	6.00±0.730	0.533±0.021	1.833±0.105	0.63±0.056	1.933±0.042

Table 2. Effect of chronic oral administration of pits of date palm on dopamine (DA) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755± 0.818	473.948±0.856	60.488±0.044	734.223±2.111	451.288±0.633	243.147±0.863
	T	200.00± 0.365**	503.000±0.856	69.833±0.307*	743.667±16.936	501.833±0.792	281.000±0.516*
	%	36.28	6.13	15.45	1.29	11.20	15.57
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	202.000±0.966**	503.000±0.856	131.6670.558***	743.667±16.936	506.167±0.477	283.500±0.428
	%	38.69	4.29	115.00	0.60	12.10	15.91

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* $p<0.05$.
** $p<0.01$.
*** $p< 0.001$.

Table 3. Effect of chronic oral administration of pits of data palm on gama-butyric acid (GABA) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.45	57.247±0.385	432.828±0.319	118.155±0.197	214.78±1.3
	T	221.500±0.428*	204.500±0.34*	64.500±0.342	435.0±0.258	201.3±0.494***	216.35±0.94
	%	15.09	19.14	12.67	0.50	70.40	0.73
2 weeks	C	192.544±0.759	171.662±0.44	57.374±0.463	432.939±0.37	117.868±0.237	214.9±1.27
	T	231.000±0.258*	224.000±0.36*	79.833±0.307**	436.83±0.48	500.167±0.307***	217.6±0.7
	%	19.97	30.49	39.14	0.90	324.35	1.26

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
% : Percentage of change from control.
* p<0.05.
** p<0.01.
*** p<0.001.

Table 4. Effect of chronic oral administration of pits of data palm on norepinephrine (NE) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997±3.242	390.050±0.831	292.540±1.536
	T	133.500±0.764**	511.673±1.912	56.855±0.276	600.000±0.365	413.667±0.667	293.243±1.263
	%	39.96	0.04	1.16	0.50	6.05	0.24
2 weeks	C	95.358± 0.857	511.118±1.648	54.443±1.898	605.330±9.485	390.490±0.484	292.527±1.531
	T	134.667±0.422**	603.000±0.856	56.688±0.307	600.167±0.307	415.167±0.703	294.433±1.184
	%	41.22*	17.98*	4.12	-0.85	6.32	0.65

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
: Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

Table 5. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755± 0.818	473.948±0.856	60.488±0.044	734.223±2.111	451.288±0.633	243.147±0.863
	T	97.167±1.621*	300.167±0.48*	44.333±0.333*	304.667±1.520**	399.500±0.428	202.167±0.872
	%	-33.79	-36.67	-26.71	-58.50	-11.48	-16.85
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	66.500±1.48**	205.167±1.33**	34.000±0.26**	155.667±1.382***	203.333±1.15**	105.667±1.4**
	%	-54.34	-57.46	-44.48	-78.94	-54.97	-56.80

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* p<0.05.
** p<0.01.
*** p<0.001.

Table 6. Effect of chronic oral administration ofmethylprednisolone (20 mg/kg b.wt.) on gama-butyric acid (GABA) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.450	57.247±0.385	432.828±0.32	118.155±0.197	214.787±1.321
	T	96.833±0.946**	99.000±0.428**	55.667±0.211	315.500±0.43*	64.500±0.764**	77.000±0.577**
	%	-49.69	-42.33	-2.76	-27.11	-45.41	-64.15
2 weeks	C	192.544±0.759	171.662±0.447	57.374±0.463	432.939±0.37	117.868±0.24	214.933±1.269
	T	65.333±0.715***	87.000±0.577**	98.500±0.428***	97.500±0.9***	84.500±0.428*	67.000±0.856***
	%	-66.07	-49.32	71.68	-77.48	-28.31	-68.83

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* p<0.05.
** p<0.01.
*** p<0.001.

Table 7. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on norepinephrine (NE) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997±3.242	390.050±0.831	292.540±1.536
	T	54.667±0.422**	301.000±0.516**	33.833±0.792*	304.833±0.703**	202.833±0.703**	101.833±0.792***
	%	-42.69	-41.15	-39.80	-48.94	-48.00	-65.19
2 weeks	C	95.358±0.857	511.118±1.648	54.443±1.898	605.330±9.485	390.490±0.484	292.527±1.531
	T	40.500±0.428**	250.500±0.342**	17.667±0.96***	201.167±0.48***	154.333±0.92***	52.500±0.764***
	%	-57.53	-50.99	-67.55	-66.77	-60.48	-82.05

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* p<0.05.
** p<0.01.
*** p<0.001.

Table 8. Effect of chronic oral administration of pits of data palm and methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus ean ± S.E.
1 week	C	146.755±0.818	473.948±0.856	60.488±0.044	739.237±4.314	451.288±0.633	243.147±0.863
	T	96.3±1.453**	297.667±0.715**	116.17±0.48***	628.8±0.6*	506.167±0.477	201.667±0.667*
	%	-34.36	-37.19	92.05	-14.93	12.16	-17.06
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	315.5±0.2***	601±0.516*	252.8±0.95***	623±0.58*	491.3±0.8	198.500±0.2*
	%	116.62*	24.61*	312.86*	-15.72*	8.81	-18.85*

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* p<0.05.
** p<0.01.
*** p<0.001.

Table 9. Effect of chronic oral administration of pits of data palm and methylprednisolone (20 mg/kg b.wt.) on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.450	57.247±0.38	432.828±0.3	118.155±0.2	214.787±1.321
	T	104.000±0.856**	122.667±0.803*	97.500±0.92***	401.000±0.5	99.0±0.4*	97.333±0.843**
	%	-45.96	-28.54*	70.31*	-7.35	-16.21*	-54.68*
2 weeks	C	192.544±0.759	171.662±0.447	57.374±0.46	432.939±0.4	117.868±0.2	214.933±1.269
	T	102.167±0.98**	206.1670.872*	99.167±0.307***	399.167±1.078	99.167±0.40*	140.000±0.931**
	%	-46.94	20.10*	72.84*	-7.80	-15.87*	-34.86*

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

Table 10. Effect of chronic oral administration of pits of data palm and methylprednisolone (20 mg/kg b.wt.) on norepinephrine (NE) content in the different brain areas of male albino rats.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997±3.242	390.050 ± 0.831	292.540±1.536
	T	133.500±0.764	511.673±1.912	56.855±0.276	600.000±0.365	413.667 ± 0.667	293.243±1.263
	%	39.96*	0.04	1.16	0.50	6.05	0.24
2 weeks	C	95.358±0.857	511.118±1.648	54.443±1.898	605.330±9.485	390.490 ±0.484	292.527±1.531
	T	134.667±0.422	603.000±0.856	56.688±0.307	600.167±0.307	415.167 ±0.703	294.433±1.184
	%	41.22*	17.98*	4.12	-0.85	6.32	0.65

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
%: Percentage of change from control.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

Table 11. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.), date palm pits and date palm pits with methylprednisolone on testosterone level in serum blood of male albino rats.

Time of decapitation		Methylprednisolone (ng/ml) mean ± S.E.	Date palm pits	Date palm pits with methylprednisolone
1 week	C	0.817±0.000	0.817±0.000	0.817±0.000
	T	0.630±0.002*	2.588±0.017***	1.381±0.003***
	%	-22.89	216.77	69.03
2 weeks	C	0.818±0.000	0.818±0.000	0.818±0.000
	T	0.640±0.003*	2.605±0.003***	1.410±0.003***
	%	-21.76	218.46	72.37

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test.

%: Percentage of change from control.

* $p<0.05$.

** $p<0.01$.

*** $p<0.001$.

Figures

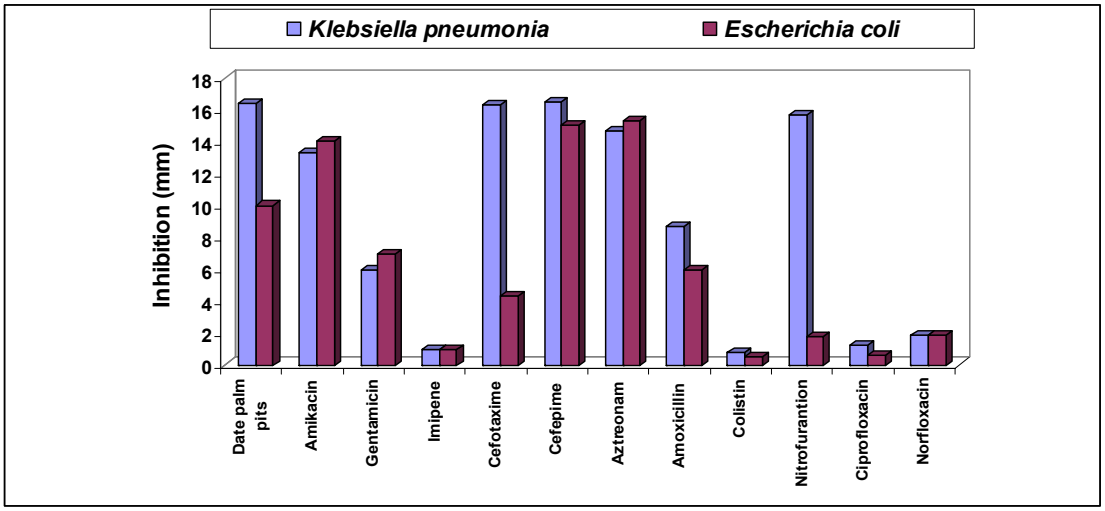

Fig. 1. Diameter of inhibition zone of the Date palm Pits (*Phoenix Dactylifera* L.) pit againt *Klebsiella pneumonia ssp pneumonia* and *Escherichia coli*.

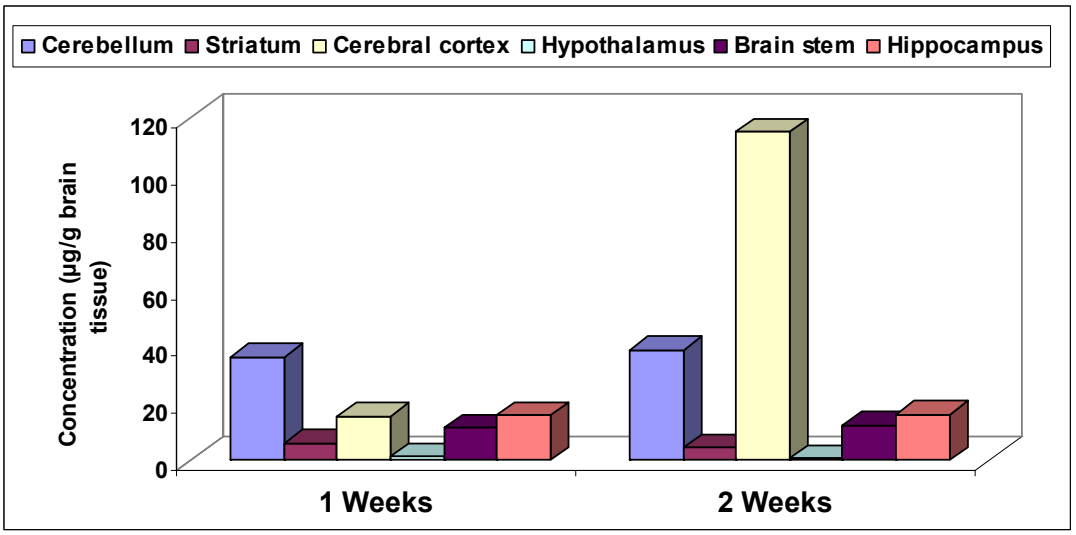

Fig. 2. Effect of chronic oral administration of pits of date palm on dopamine (DA) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

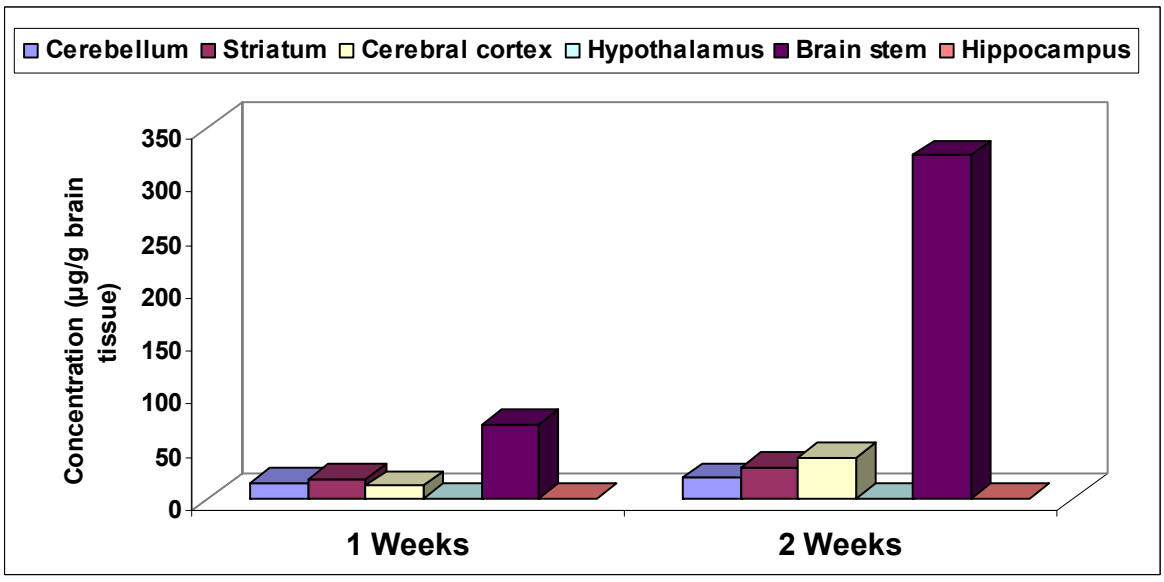

Fig. 3. Effect of chronic oral administration of pits of date palm on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

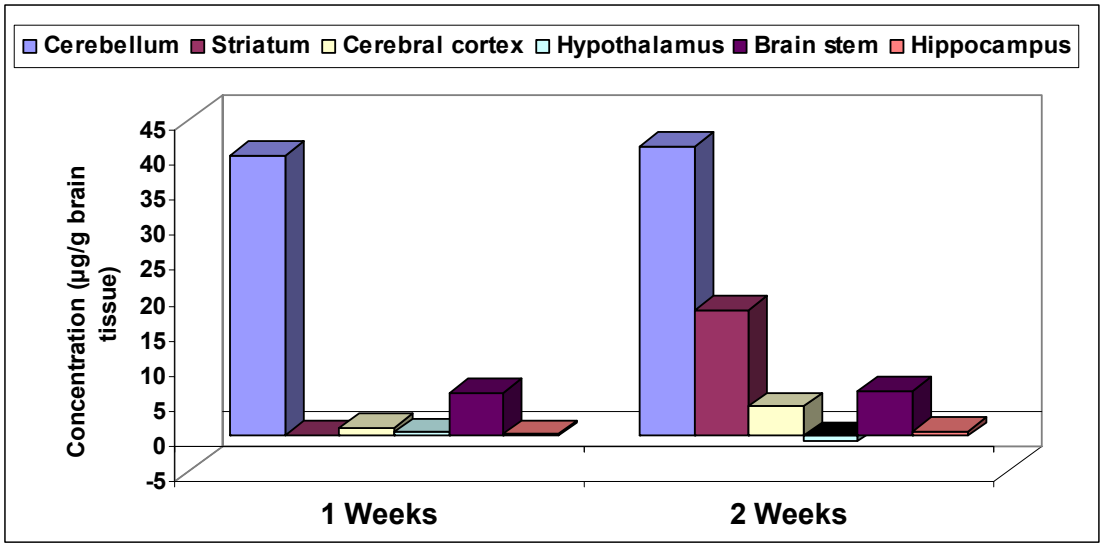

Fig. 4. Effect of chronic oral administration of pits of date palm on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

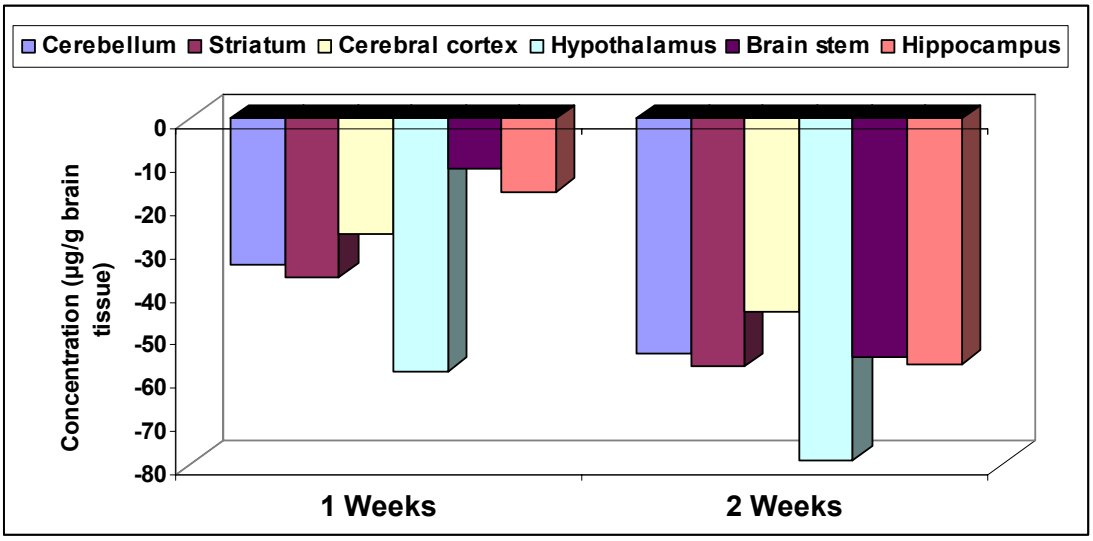

Fig. 5. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

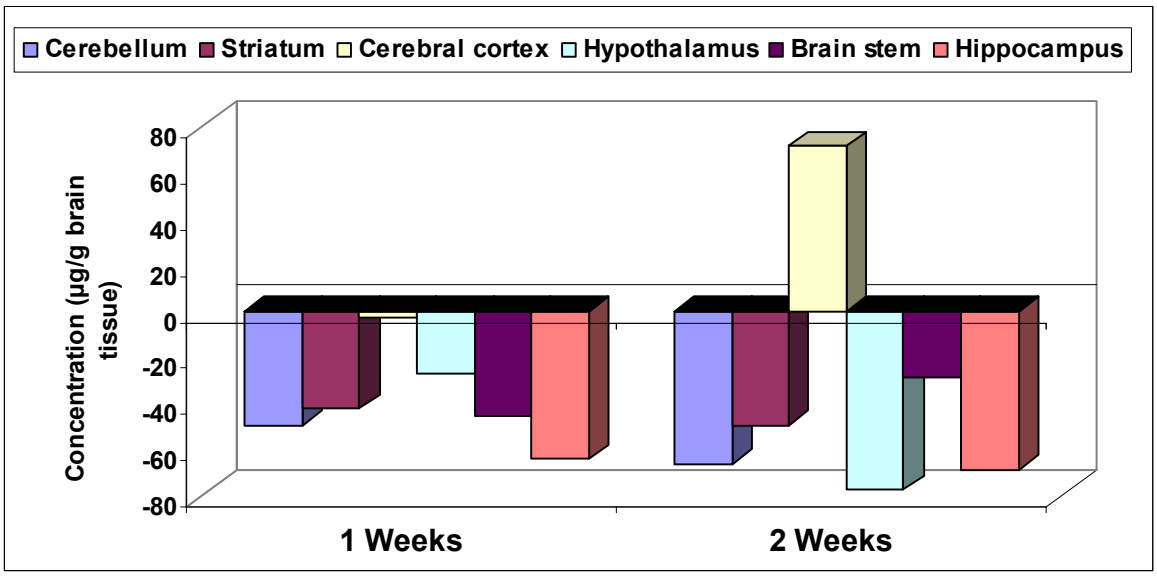

Fig. 6. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

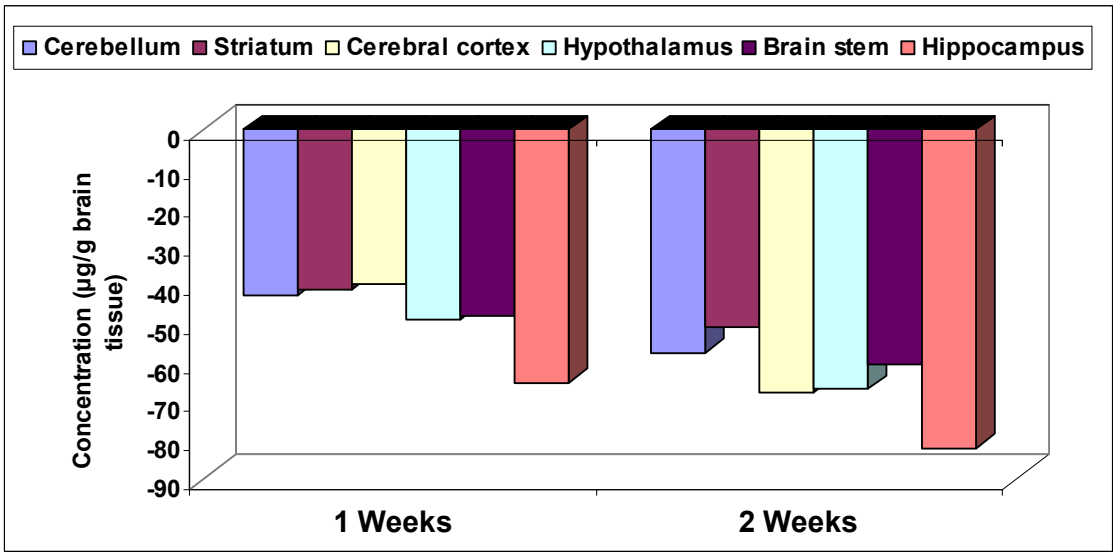

Fig. 7. Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino rat.

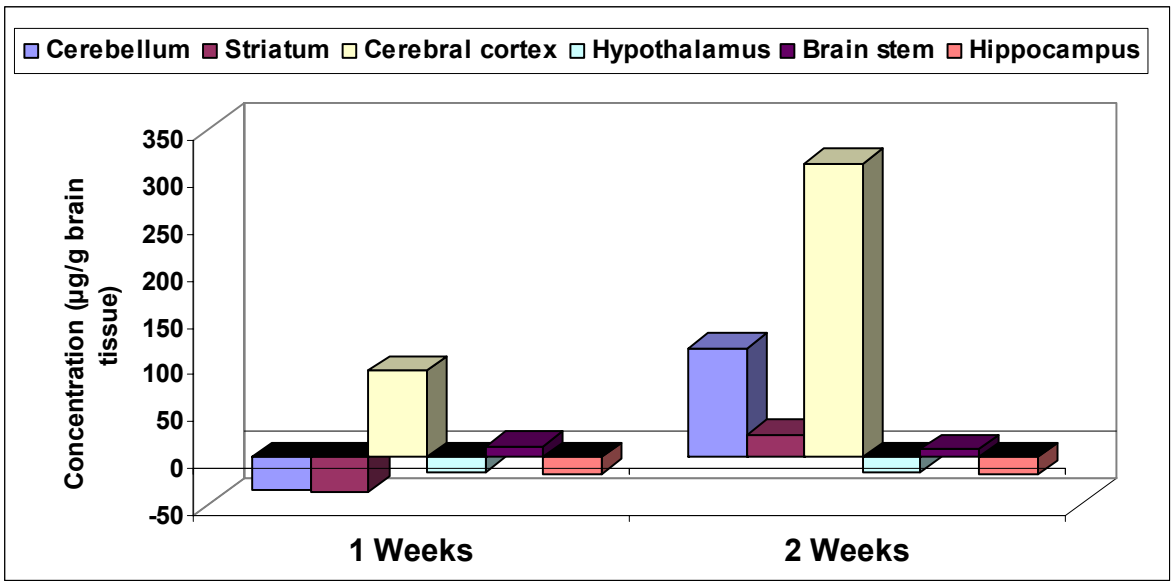

Fig. 8. Effect of chronic oral administration of pits of date palm and methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content represented by the % difference between control and treated values in the different brain areas of male albino rats.

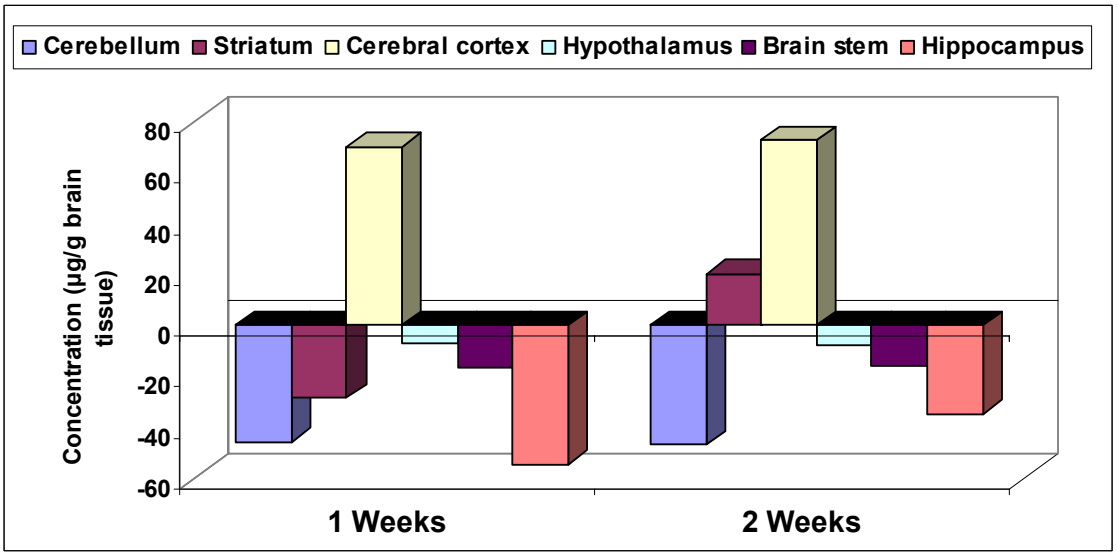

Fig. 9. Effect of chronic oral administration of pits of date palm and methylprednisolone (20 mg/kg b.wt.) on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of male albino rats

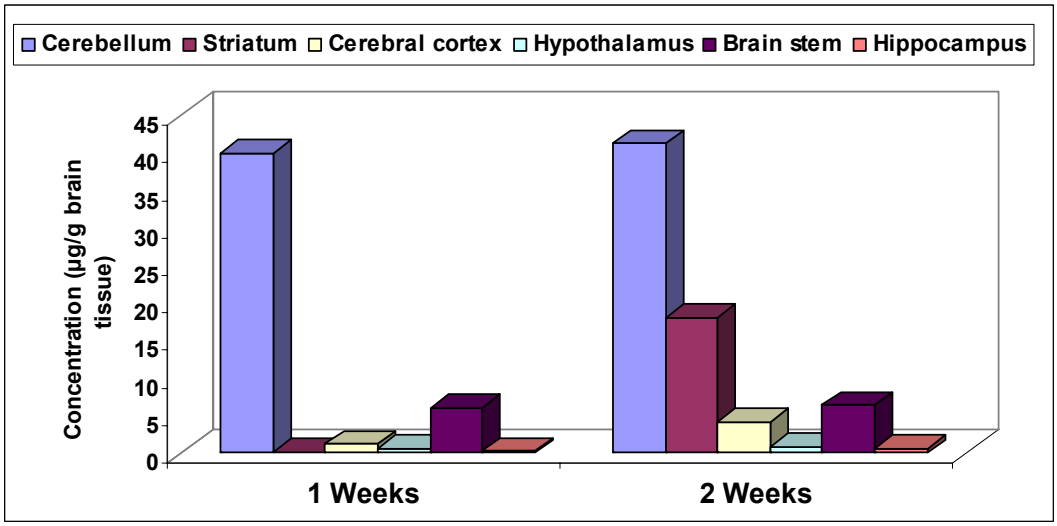

Fig. 10. Effect of chronic oral administration of pits of date palm and methylprednisolone (20 mg/kg b.wt.) on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino.

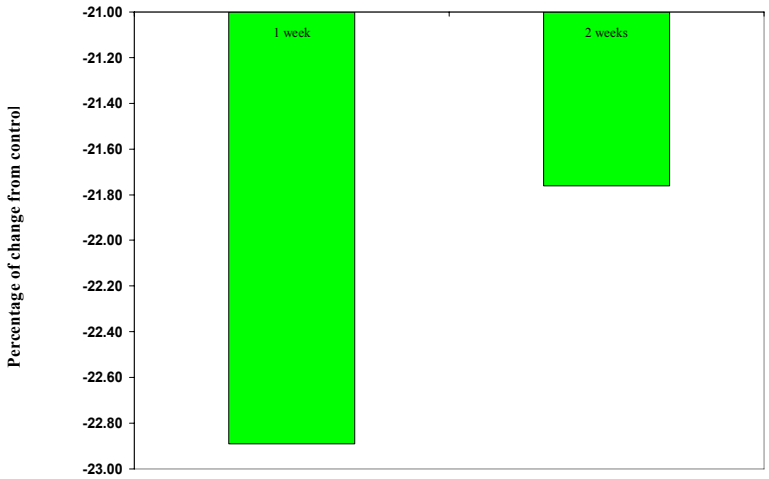

Fig. 11. Effect of chronic oral administration of methylprednisolone on testosterone level represented by the % difference between control and treated values in serum of male albino rats.

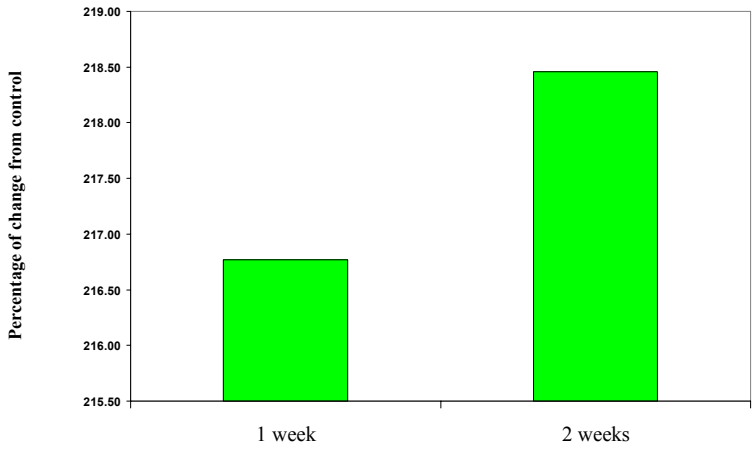

Fig. 12. Effect of chronic oral administration of pits of date palm on testosterone level represented by the % difference between control and treated values in serum of male albino rats.

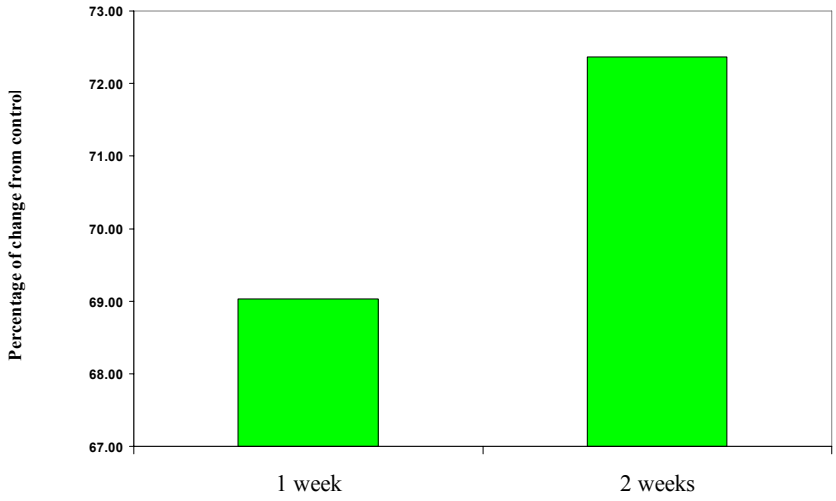

Fig. 13. Effect of chronic oral administration of date palm pits with methylprednisolone on testosterone level represented by the % difference between control and treated values in serum of male albino rats.

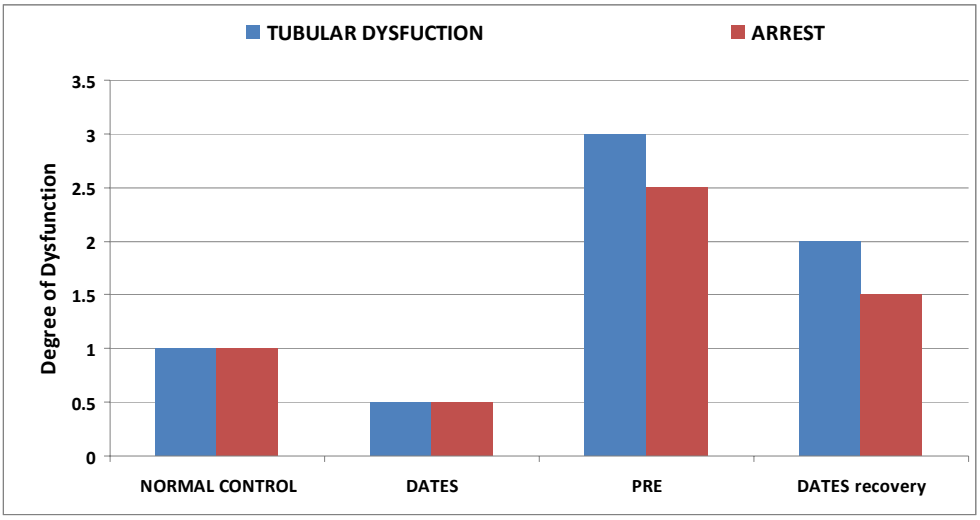

Fig. 14. The effect of pits of data palm enhanced spermatogenesis, versus normal control and methylprednisolone (pre). Notice a significant enhanced spermatogensis induced by dates when compared by normal & methylprednisolone effects. Dates recovery = pits of data palm and methylprednisolone, PRE = methylprednisolone Tubular dysfunction and arrest.

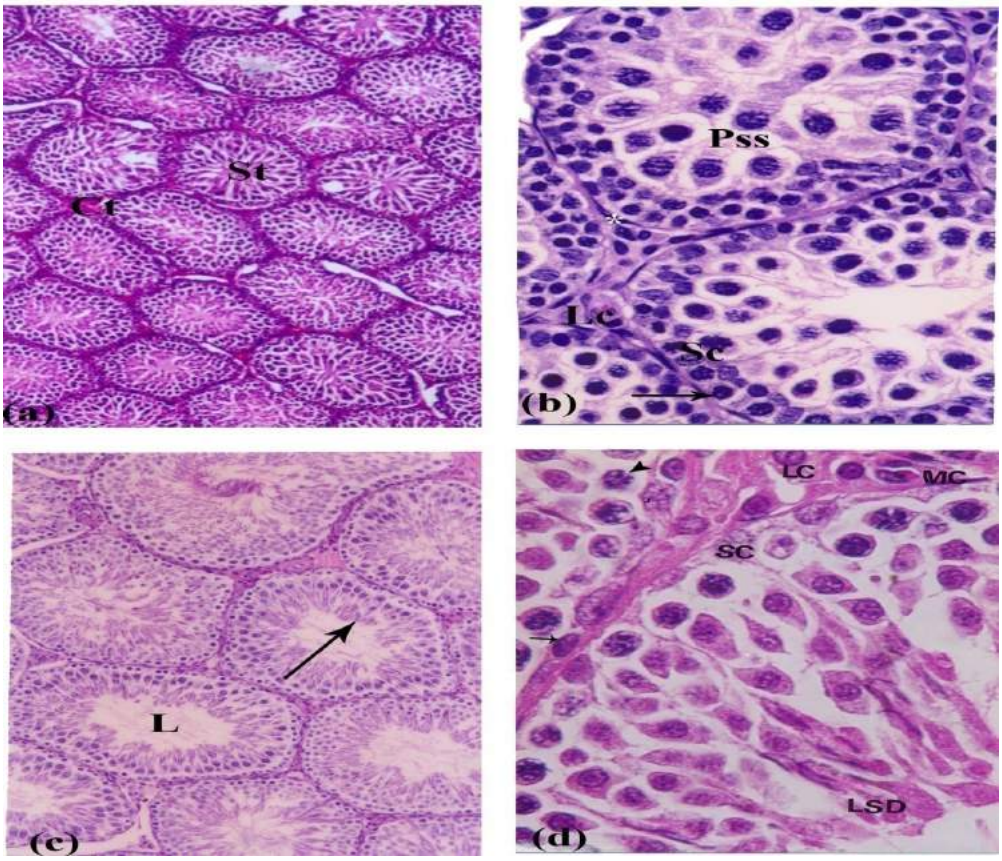

Plate 1a-d. Transverse sections of tests of male rate for control group (G_1). 1a: note small seminiferous tubules (St) mostly without lumen, surrounded by fibrous connective tissue (CT) layer (H and E; ×100). 1b: High magnification showing dark spermatogonium (↗) and pachytene spermatocytes (Pss) and Sertoli cell (SC). Note, myoid cell (*) Layer surrounded St and intertubular space contains leydig cells (Lc) (H & E, ×400).1c: Note, enlarged seminiferous tubules populated by spermatocytes and late spermatids (↗) surround the tubular Lumen (L) (8 weeks of age (H and E; ×100). 1d: High power from 2c showing Light (▲) and dark (↗) spermatogonium adjacent to basal Lamina; late spermatids (LSD) with elongated head directed towards Sertoli cells (Sc). Note, myoid cell (Mc) and Leydig cells (LC)

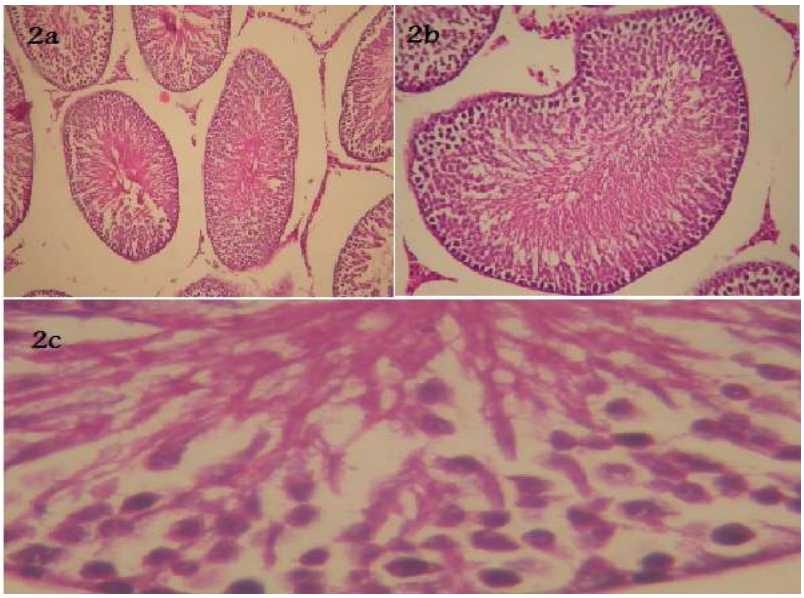

Plate 2. Effect of chronic oral administration of pits of data palm on testicles of male albino rat. 2a. A distented tubules showing an increased active spermatogenesis with significant rise of number of mature sperms (H and E; ×40). 2b. tubule showing an increased active spermatogenesis (H and E; x 100). 2c. Marked increase in spermatogenesis, free of early or late arrest. (H and E; ×400)

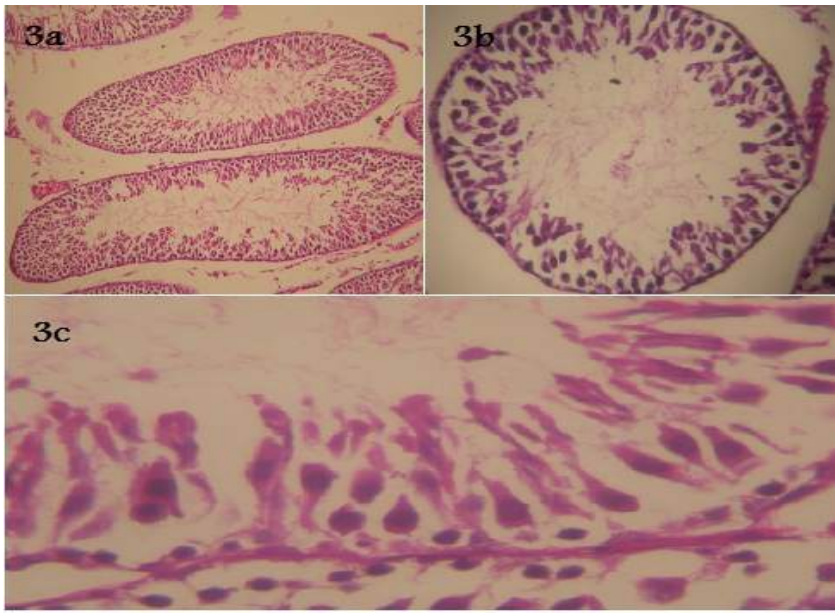

Plate 3. ffect of chronic oral administration of methylprednisolone on testicular of male albino rat. 3a. A distented tubules showing Marked reduction of spermatognesis (hypospermatogenesis) (H and E; ×100) 3 b. tubule showing Partial late arrest with marked reduction of mature sperms (H and E; ×100). 3c. Early arrest with absent mature sperms & germ cell hypoplasia (H & E; ×400. 3d. There are focal areas of disrupted architecture & the tubules show absent spermatogenesis (H & E; ×1000). 4e. Foci of interstitial fibrosis, congestion, vascular injury 'endartritis" and inflammation (H and E; ×400).

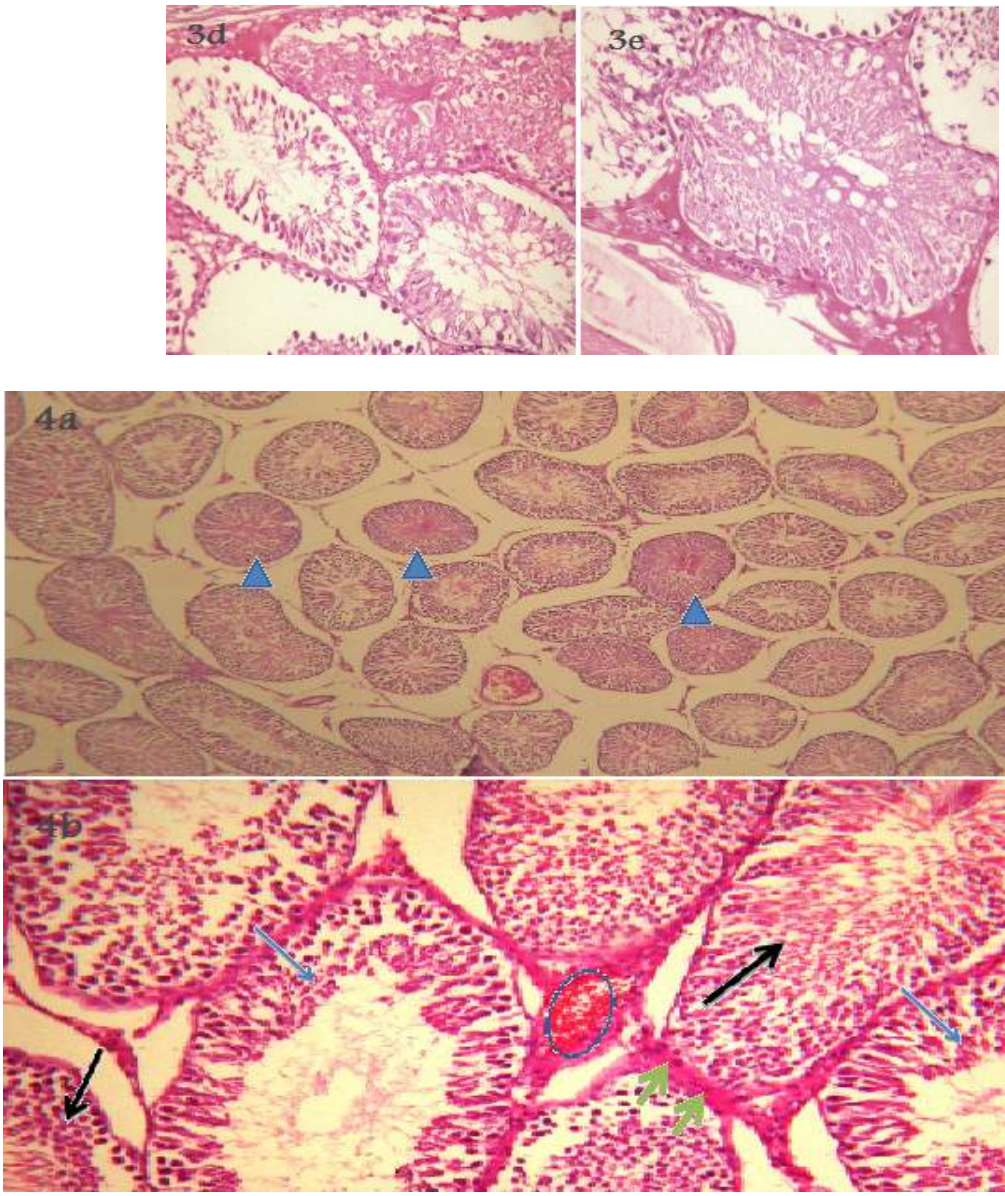

Plate 4. Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat. 4a. Recovery effect (H and E; ×40). Notice high sperm in some tubules (arrows). 4b. There is partial tissue recovery. Tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules (blue arrows and cycle). Intact tubules varies from hypospermatogenesis to near normal count (black arrows). There is minimal interstitial fibrosis (green arrows).

Evaluating Utilization of Ground Date Stone with or without Kemzyme in the Diets of Growing New Zealand Rabbits

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Keywords: ground date stone, kemzyme, growth, performance, digestion, carcass traits

Abstract

A total number of 45 New Zealand white weaned male rabbits aged 30 days with nearly equal live body weights was used in the present study to determine the effects on growing rabbits' growth performance and carcass traits when replacing yellow corn content of the basal diet by ground date stone either partially (50% replacement) or totally (100%). Each replacement included two treatments, with or without kemzyme supplementation. Five groups were randomly allotted with three replicates of 3 rabbits each. Five pelleted experimental diets were formulated to be approximately iso-nitrogenous. Treatment diets were formulated by replacing 50 or 100% of yellow corn in the control by ground date stone with or without kemzyme (at a level of 0.5 kg/ton of rabbit feed). The results indicated that 50% replacement of yellow corn ground date stone (with or without kemzyme) significantly (P<0.05) improved feed conversion efficiency of growing male rabbits during the experimental period. Replacing yellow corn at 50 or 100% by ground date stone with kemzyme supplementation slightly improved digestibility coefficients of dry matter, crude protein, crude fiber, ether extract and NFE. The dressing percentage was significantly (P<0.05) higher with supplementing kemzyme to at 50 or 100% ground date stone, compared to the other experimental groups. The obtained results indicated that ground date stone could be used instead of yellow corn (50% substitution) either without or with special kemzyme supplementation which showed best performance and economic efficiency.

In general, the results indicated that ground date stone could replace yellow corn in rabbits feed by 50% either with or without kemzyme, to realize the best production and economic efficiency values.

INTRODUCTION

The prices of the main ingredients of feedstuffs used in formulating poultry and rabbits feed are spontaneous increasing, besides most of them are imported. So it is of benefit to apply new unconventional local sources of low price. Rabbits are herbivores and consume high fiber diets. They are hind-gut fermenters and are capable of retaining small fiber particles for digestion (Ehrhein et al., 1983). A higher intake of a fibrous diet is achieved when nutrient requirements are met by digestibility of the non-fiber component (Hintz et al., 1978). The digestive strategy of rabbits for the utilization of fibrous diets was described by Cheeke (1987). Rabbits can separate fiber and non-fiber components and retain non-fiber components for fermentation in the cecum. The ground date stone as a cheap by-product of date fruit crops was purchased to be experimented on in this study.

This work aimed to determine the effects on growing rabbits' growth performance and carcass traits when replacing the yellow corn content of the basal diet by ground date stone either partially (50% replacement) or totally 100%. Each replacement included two treatments, with or without kemzyme supplementation.

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MATERIAL AND METHODS

The experimental work of this study was carried out at the Poultry Nutrition Farm, Animal Production Department, Faculty of Agriculture, Cairo University, during winter 2003.

Five pelleted experimental diets were formulated to be approximately iso-nitrogenous. The diets were formulated to cover the requirement of growing rabbits according to NRC (1977) and Cheeke (1987) but with replacement of yellow corn (at 50 or 100%) by date stone meal with or without kemzyme at level of 0.5 kg/ton of rabbit feed (Table 1). Kemzyme compound is a multiple enzyme product. Each gram comprises alpha amylase 400 unit, cellulase complex 400 unit, beta glucanase 1250 unit, protease 450 unit, lipase 100 million unit and xylanase 20000 unit.

Forty-five New Zealand white weaned male rabbits of five weeks of age and having approximate equal live body weights were randomly allotted to five groups with three replicates of 3 rabbits each (Table 2). Rabbits of each replicate were housed in separate cages and kept under the same managerial hygienic condition. Diets were offered to the rabbits ad libitum and fresh water was available all the time during the experiment. Individual live body weight, feed intake and feed conversion ratio were recorded weekly. Digestibility trails were carried out using four male rabbits from each experimental group at the last week of the experiment. Rabbits for each group were housed in metabolism cages where feces and urine were collected separately for four consecutive days. Proximate analysis of the diets and feces were carried out according to the methods of AOAC (1990). At the end of the experimental period (13 weeks of age), four rabbits were randomly taken from each group and fasted for 12 hours before slaughter according to Blasco et al. (1993). The economical efficiency (EE) was calculated according to the following equation:

$$EE = (A\text{-}B/B) \times 100$$

Where, A is the selling price of obtained gains and B is the feeding cost for these gains in Egyptian pound (L.E).

All data were subjected to analysis of variance using the general linear models (GLM) procedure of SAS (1994) and differences obtained upon statistical analysis were compared using the Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical analysis composition of the experimental diets and the ingredients used in its formulation is presented in Table 3.

The effects of the experimental diet on body weight, body weight gains, feed intake and feed conversion values of growing male rabbits during the experimental period (5-13 weeks of age) are shown in Table 4. The results indicated that feeding growing rabbits on diets containing 10% ground date stone (50% replacement of yellow corn content) and either supplemented or not with kemzyme had significantly ($p<0.05$) improved both body weight gain and feed conversion efficiency compared to those fed the totally replacement of yellow corn by ground date stone diets. The obtained values were 1055, 888, 1300 and 685 g for body weight gain and 2.80, 4.17, 3.18 and 4.98 for feed conversion efficiency, respectively. On the other hand, feeding growing rabbits on diets T3 and T4 supplemented with kemzyme when containing ground date stone the two levels of substitution resulted in an improvement in feed conversion efficiency when compared to those fed diets without kemzyme. The values were 3.18 and 2.80 for T2 and T3 respectively in which the diets contained 10% ground date stone. However, the values were 4.98 and 4.17 for T44 and T5 respectively in which the diets contained 20% ground date stone. The improvement in rabbits' performance may be due to the beneficial role of kemzyme in improving the digestibility of experimental diets. The results herein were supported by those reported by Mukhametagoliev et al. (1986), El-Katasha et al. (1988) and Tawfeek (1996) who found that supplemented diets with kemzyme improved growth

performance of growing rabbits fed diets containing corn or barley grains.

Generally, the best feed conversion was recorded with the rabbits group fed 50% ground date stone diets supplemented with kemzyme, compared to the other experimental groups.

Digestibility and Nutritive Values

Results in Table 5 show that substitution of 50% yellow corn by ground date stone in growing rabbits diets and supplemented with kemzyme slightly increased the digestibility coefficients of DM, CP, EE, CF and NFE compared to the other experimental diets. Generally, the highest digestibility of all nutrients was recorded with rabbits fed diets containing 10% ground date stone (50% replacing the yellow corn content) and supplemented with kemzyme.

These results somewhat agreed with those obtained by Osman et al. (1996) and Attia et al. (1998) who found that supplementing pekine ducklings diets containing barley with multi-enzyme improved the digestibility of fat and protein. Besides, Aboul-Ela et al. (1999) reported that DM, CP and EE digestibility slightly improved when growing New Zealand white rabbits fed date pits at a level of 5%, compared to those fed the control diet. They also noted that the nutrients digestibility significantly ($p<0.05$) decreased as the date pits level increased up to 100%.

The previous results obtained on nutrients digestibility and feeding value of the diets revealed that substitution of 50% yellow corn by ground date stone with kemzyme in growing rabbits diets achieved the highest digestibility and nutritive value compared to the other experimental groups. The benefit gained by addition of enzyme may be due to the largely partial degradation of soluble beta-glucan in barley and corn diets, reducing the viscosity of intestinal contents and improving nutrient absorption (Hesselman and Aman 1986). In this respect Zatari and Ferket (1990) revealed that the hydrolytic action of enzyme as a mixture including glucanase, gulactosidase, proteinase and cellulase works synergistically to improve the nutritive value of a diet.

Carcass characteristics at 13 weeks of age are presented in Table 6. Dressing percentage of rabbits meat was significantly ($p<0.05$) higher with feeding on diets in which kemzyme was supplemented and ground date stone replaced its content of yellow corn at 50% or 100%. However, giblets percentages of rabbits meat were insignificantly affected among the experimental groups. With regard to chemical analysis of meat, no significant differences were noticed in both crude protein and moisture percentage, while the ether extract percentage of meat was significantly lower when growing rabbits fed 20% date stone meal without kemzyme, compared to those rabbits fed the other experimental diets.

These results agree with those reported by Toson et al. (1995) who found that dressing percentage of rabbits fed on diets containing date stone meal up to 100% (without kemzyme) was approximately to that of the control diet. Further studies carried out by Onwudike (1986) and Brufau (1991) on broilers and Fetuga et al. (1977) on pigs, showed that replacement date stone meal in diets did not significantly affect carcass traits, while carcass fat was significantly reduced with increasing date stone meal.

Economical Efficiency

Results in Table 7 show that the profitability of using ground date stone as a partial or complete substitution for yellow corn in rabbit diets depends on the price of this feedstuff, assuming that the other costs are constant. Therefore, the economic efficiency of feeding diets at marketing age (13 weeks) was only higher with replacing yellow corn by 10% ground date stone without kemzyme compared to the other experimental diets.

Conclusively, the obtained results indicated that ground date stone can be used at the level of 10% in rabbit diets (replacing yellow corn at 50% level) either not supplemented or supplemented with kemzyme for best performance and economic efficiency.

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Tables

Table 1. Ingredients and chemical analysis of the experimental diets.

Ingredients (%):	Experimental diets				
	D1	D2	D3	D4	D5
Yellow corn	20	10	10	-	-
Ground date stone	0	10	10	20	20
Wheat bran	28	28	27	28	27.95
Soy bean meal (44% CP)	17	17	17	17	17
Clover hay	33.2	33.2	33.2	33.2	33.2
Methionine	0.1	0.1	0.1	0.1	0.1
Lime stone	1.0	1.0	1.0	1.0	1.0
Salt	0.4	0.4	0.4	0.4	0.4
Kemzyme	-	-	0.05	-	0.05
Vit & min. premix*	0.3	0.3	0.3	0.3	0.3

* Vitamin and mineral premix at 0.3% of diet supplies the following per kg of diet: Vit. A 1200 IU, ; 500.000 IU.D3; 0.67 mg Vit.K3;0.67 mg Vit B1; 2.0 mg Vit.B2; 0.67 mg Vit.B6; 0.0004 mg Vit.B12; 16.7 mg Pantothenic acid; 0.07 mg Biotin; 1.67 mg Folic acid; 400 mg Choline chloride; 22.3 mg Zn; 10 mg Mn; 25 mg Fe; 1.67 mg Cu; 0.25 mg I; 0.033 mg Se and 133.4 mg Mg.

Table 2. The experimental treatments of the study.

Treatment	R*	Rabbits/R	Diets fed	Replacement	Kemzyme supplementation
T1	3	3	D1	No (control diet containing 20% yellow corn)	Without
T2	3	3	D2	Replacing (50%) of the yellow corn by ground date stone	Without
T3	3	3	D3	Replacing (50%) Of the yellow corn by ground date stone	With kemzyme supplementation
T4	3	3	D4	Total replacing (100%) of the yellow corn by ground date stone	Without
T5	3	3	D5	Total replacing (100%) of the yellow corn by ground date stone	With kemzyme supplementation

Table 3. Chemical analysis of used ingredient and experimental diets.

Item	Experimental treatment						
	T1	T2	T3	T4	T5	Yellow corn	Ground date stone
DM	86.73	86.76	86.72	86.79	86.75	89.0	89.3
OM	94.59	94.40	94.41	94.21	94.22	98.5	96.8
CP	17.32	17.12	17.11	16.92	16.91	9.3	6.0
CF	12.74	13.93	13.92	15.12	15.11	2.2	13.9
EE	4.47	4.05	4.05	3.63	3.63	4.4	7.8
Ash	5.41	5.60	5.59	5.79	5.78	1.5	3.2
NFE	60.07	59.31	59.32	58.55	58.56	82.6	69.0

Table 4. Growth performance of New Zealand white rabbits as affected by replacing yellow corn with ground date stone with or without kemzyme supplement during the growing period from 5 to 13 weeks of age.

Item	Experimental treatment					MSE
	T1	T2	T3	T4	T5	
Initial body weight (g)	695	690	695	685	695	1.75
Body weight at 13 weeks (g)	1845^{a}	1990^{a}	1750^{ab}	1370^{c}	1583^{bc}	64.62
Total body weight gain	1150^{a}	1300^{a}	1055^{ab}	685^{c}	888^{bc}	64.11
Total feed intake	3843^{ab}	4123^{a}	2875^{c}	3413^{bc}	3600^{ab}	135.59
Feed conversion	3.34^{bc}	3.17^{c}	2.73^{c}	4.98^{a}	4.05^{ab}	0.24

a,b,c Means values in the same row bearing different letters differ significantly (P<0.05).

Table 5. Nutrient digestibility and nutritive value of New Zealand white rabbits as affected by replacing yellow corn with ground date stone with or without kemzyme supplementation.

Item	Experimental treatments					MSE
	T1	T2	T3	T4	T5	
Digestibility						
Dry matter (DM)	74.84	73.58	76.10	68.63	76.92	1.26
Crude protein (CP)	79.46	78.18	81.82	76.49	78.84	1.01
Crude Fiber (CF)	40.70	40.05	45.06	39.30	46.56	1.71
Ether extract (EE)	64.99^{ab}	61.62^{b}	69.10^{a}	63.00^{ab}	66.12^{ab}	1.00
Nitrogen free extract (NFE)	86.11^{a}	84.98^{ab}	86.20^{a}	79.43^{b}	86.52^{a}	1.00

a,b,c Means values in the same row bearing different letters differ significantly (P<0.05).

Table 6. Carcass traits and chemical analysis of New Zealand White rabbits as affected by replacing yellow corn with ground date stone with or without Kemzyme supplementation.

Item	Experimental diets					MSE
	T1	T2	T3	T4	T5	
Dressing (%)	53.83	53.52	59.62	54.97	59.01	1.16
Giblets (%)	4.42^{ab}	4.31^{ab}	4.23^{b}	4.44^{ab}	5.01^{a}	0.11
Chemical analysis of meat:						
Moisture (%)	74.25	73.87	75.46	74.21	74.17	0.27
Crude protein (%)	20.67	20.74	21.59	20.54	20.61	0.27
Ether extract (%)	5.65^{ab}	5.23^{bc}	6.01^{a}	5.05^{c}	5.83^{a}	0.12

a,b,c Means values in the same row bearing different letters differ significantly (P<0.05).

Table 7. Economic efficiency of the experiment treatments from 5 to 13 weeks of age.

Item	Experimental treatment				
	T1	T2	T3	T4	T5
Number of survival rabbits	9	9	9	8	9
Average feed intake/rabbit (kg)	3.843	4.123	2.875	3.413	3.600
Total feed intake (kg)	34.59	37.11	25.88	27.30	32.40
Price/kg feed (L.E.)	0.93	0.88	0.90	0.83	0.85
Total feed cost (L.E.)	32.17	32.66	23.29	22.66	27.54
Average body weight gain (kg)	1.150	1.300	1.055	0.685	0.888
Total meat yield (kg)	10.35	11.70	9.50	5.48	7.99
Selling price* (L.E.)	113.9	128.7	104.5	60.28	87.89
Economic efficiency**	81.68	96.04	81.21	37.62	60.35
Relative economic efficiency	100	117.58	99.42	46.06	73.89

* Selling price of 1 kg=11 L.E.
** Economic efficiency = selling total meat yield - total feed cost.

A Comparative Study on Date Syrup (Dips) as Substrate for the Production of Baker's Yeast (*Saccharomyces cerevisiae*)

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Keywords: date dips, Dips, molasses, baker's yeast, biotin, pantothenic acid, substrates, formic, propionic

Abstract

The suitability of date syrup (Dips) as a substrate for the production of baker's yeast (*Saccharomyces cerevisiae*) was examined and compared with molasses substrate as a reference. With regard to nutrient content, Dips compares very well with molasses in this respect. Dips contains more sugars, biotin and pantothenic acid than molasses, about similar contents of nitrogen, phosphorus and magnesium, about half the content of potassium (but still enough for baker's yeast production), and much less m-Inositol. Like molasses, Dips contains some compounds toxic to *Saccharomyces cerevisiae* including formic, acetic and propionic acid, while other toxic compounds occasionally found in molasses such as nitrite, sulfite and butyric acid were not detected in Dips. Addition of molasses to Dips at 1:1 ratio will bring the end concentration of formic acid to values below toxicity level. Hence, Dips can be used at 50% of the substrate for production of baker's yeast, which is a reasonable amount of utilization.

INTRODUCTION

The annual production of date fruits in Saudi Arabia is estimated at about 712,000 tons. It has been increasing steadily in the last ten years at a rate of about 1.9% every year (Al-Eid et al., 2009). All amounts of dates consumed locally as human food or exported to foreign markets make only about 50% of the annual production. A small part of the remaining produce is used as animal feed while the larger part is a surplus waiting for suitable means of economic utilization (Al Obaidi et al., 1985). Baker's yeast can be produced from substrates that contain metabolizable sources of carbon, energy, nitrogen, minerals and essential vitamins. Potential substrates range from food materials like grains and dates, by-products of the food industry like molasses and whey, and wastes like agricultural residues and food rests. Substrates that contain carbon and energy sources readily metabolizable for *Saccharomyces cerevisiae* such as mono- and disaccharides in dates and molasses can be directly used for production, while those containing complex carbohydrates such as starches in grains and cellulose in plant rests need costly hydrolytic treatments before use. The substrate of choice for baker's yeast production in the world today is molasses.

Commercial baker's yeasts produced from strains of *Saccharomyces cerevisiae* have the following average chemical composition: 47% C, 32% O_2, 6% H_2, 7.7% N_2, 2% K, 1.2% P, 1% S, 0.2% Mg, 0.1% Na, and other trace elements. In addition, the yeast cells contain small amounts of vitamin B complex, of which D-Pantothenic acid, D-Biotin and m-Inositol are essential because the yeast cells cannot synthesize them (Al Obaidi et al., 1987). These elements and compounds must be provided in the production medium in enough quantities and metabolizable forms.

Dates are supposed to make a good potential substrate for baker's yeast production serving mainly as carbon and energy source. According to Sawaya (1986), dates contain 65-87% sugars, 1-3% proteins, in addition to many minerals important for yeast nutrition

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including potassium, magnesium, sulphur, phosphorus, iron, calcium and chlorine.

Few investigations about the production of baker's yeast from date extracts have been conducted (Al-Eid et al., 2009; Al Obaidi et al., 1985, 1986, 1987; Khan et al., 2000; Mudhaffer, 1978; Nancib et al., 1997). Although most workers described dates as satisfactory for baker's yeast production, the yields they obtained were very low. A maximum of about 43% of the theoretical yield and only 10.7 g/L biomass concentration was reached in the fermentation medium compared to the optimum of about 40 g/L expected for an economical production. Al Obaidi et al. (1985, 1986, 1987) reported an average of 47% of the theoretical yield, and Nancib et al. (1997) reached a maximum of 0.6 g/L biomass concentration in the fermentation medium.

The aim of this present study was to determine the chemical composition of date syrup (Dips) and compare it with molasses as substrates for baker's yeast production.

MATERIALS AND METHODS

Date Syrup (Dips) Extraction

Extraction of Dips used in this study was carried out using the technology adopted in a date processing factory in AL Hofuf City as follows: Date ('Ruzeiz' variety) was de-pitted and the flesh heated with equal amount of water at 80°C for 30 min. The mixture was filter pressed to remove large impurities and insoluble matters, then micro-filtered to remove smaller impurities and obtain a clear extract using a NOVOX 200 sheet filter system (Filtrox AG, CH 9000 St. Gallen, Switzerland) with an effective clarifying filtration area of 1.02 m^2 and 2.0 μm pore size, operated at a pressure of 1 bar. Finally the extract was concentrated to 75°Brix at low temperature (80ºC) by vacuum drying to give the syrup named Dips.

Chemical Analysis

Phosphorus, potassium, magnesium, ash, protein, nitrite and sulfite were determined according to AOAC standard methods of analysis (AOAC, 1992).

Formic, acetic, propionic and butyric acids were determined using Gas Liquid Chromatography, Column: LiChroCART 125-4, Purospher RP-18 e, 5 μm; mobile phase: A: 20 mM sodium dihydrogen phosphate, pH 2.5; B: Acetonitrile; Detection: UV 220 nm.

Biotin and pantothenic acid were determined according to the vitaFast (r-biopharm) vitamin testing method, using an ELISA Reader, Multiskan EX Model No. 355, Thermolabsystem-Finlan.

Sucrose, glucose, fructose and m-Inositol were determined with High Performance Liquid Chromatography (HPLC) using a Shimadzu Japan Model 2003, equipped with a RID 10A refractive index detector, CLC NH_2 6×150 column, LC 10ATP pump, and CTO 10AC VP oven. Mobile phase was 83% CH_3CN:17% water (v/v), flow rate 1 ml/min, column pressure 200 kgF/cm^2.

Treatment of Dips

To try to remove the volatile fatty acids contents in Dips which represents the toxic substance for baker's yeast, a treatment similar to the one performed for molasses clarification in baker's yeast factories (for the removal of similar compounds) was performed as follows: Dips was diluted 1:3 with water, pH brought to 4.5 with sulfuric acid, and then it was boiled in an open container under continuous aeration for about 15 min.

RESULTS AND DISCUSSION

Chemical Composition of Dips as Substrate for Baker's Yeast Production

Dips was chemically analyzed to determine its contents for nutrients needed by baker's yeast (*Saccharomyces cerevisiae*) and also to determine the presence of chemicals that could be toxic to the yeast. Results presented in Table 1 show that Dips contained

about 80% total sugars, i.e., 800 kg/ton, mainly in the form of fructose (41%), and glucose (38%), and a small amount of sucrose (1%). It also contained 2% crude protein, 1.13 g/kg phosphorus, 14.88 g/kg potassium, 0.79 g/kg magnesium, 240 ppm pantothenic acid, 2.73 ppm biotin and no detectable amounts of m-Inositol.

These results indicate that Dips can be used as a main source of carbon and energy for baker's yeast production. Calculations based on the chemical composition of Dips shown in Table 1 give the following: since a tone of Dips contains 800 kg sugar, then this tone, regarded as carbon and energy source, should produce about 400 kg dry yeast, because one kg sugar is known to yield about 0.5 kg yeast dry matter (Bronn, 1990; Bailey and Ollis, 1986), i.e., we need about 2.5 kg Dips for every kg yeast dry matter produced. Protein content of Dips is about 2%, i.e., 20 g/kg, whereas baker's yeast contains about 50% proteins, i.e., 500 g/kg yeast dry matter. This means that one kg yeast (i.e., 500 g protein) contains about 80 g nitrogen (16% nitrogen in proteins), and the content of 2.5 kg Dips needed as carbon and energy source to produce one kg yeast (see above) is about 8 g nitrogen, hence a deficiency of about 72 g nitrogen for every kg yeast produced. This nitrogen deficiency in Dips must be covered by adding to the production medium inorganic nitrogen sources such as ammonium salts. Phosphorus content in Dips is 1.13 g/kg (about 2.8 g/2.5 kg Dips), compared to 14.1 g/kg in the yeast, i.e., Dips is deficient in phosphorus which has to be added to the production substrate in form of inorganic phosphorus at about 11.5 g phosphorus for every kg yeast produced. Magnesium content in Dips is 0.79 g/kg (about 2 g/2.5 kg Dips), and its content in baker's yeast is 2 g/kg, meaning that it is just enough. No m-Inositol was detected in Dips, thus about 2 g of this compound must be added to the production medium for every kg yeast produced. The content of Dips of potassium is about 15 g/kg (37 g/2.5 kg Dips), whereas the content of yeast from this chemical is about 20 g/kg yeast dry matter. This means that the content of potassium in Dips is enough for baker's yeast production. Similar calculations show that biotin and pantothenic acid are present in Dips in quantities enough for baker's yeast production.

To compensate for the deficiencies in nutrients mentioned above, mineral media with compositions as shown in Table 2 were used in our laboratories for the production of up to 40 g/L yeast dry matter (e.g., 400 g yeast in a 10-L fermentation volume). The substrates used were pure Dips, pure molasses, and 1:1 Dips: molasses. The molasses and Dips/molasses substrates gave satisfactory and comparable yields, while the yields from pure Dips were unsatisfactory (Al-Eid et al., 2009). The reduced yields from pure Dips substrate were attributed to the presence of compounds toxic to the yeast in Dips.

Comparing Dips and Molasses as Substrates for Baker's Yeast Production

With regard to its content for nutrients, Dips can compare very well with molasses which is the conventional substrate for baker's yeast production world wide (Table 3). Dips contains much more sugars, biotin and pantothenic acid than molasses, about similar contents from nitrogen, phosphorus and magnesium, about half the content of potassium (but, still enough for baker's yeast production), and much less m-Inositol. Compounds toxic to baker's yeast detected in Dips include formic acid at 3.06%, acetic acid at 2.38%, and propionic acid at 0.68%, (total acids 6.12%), and no detectable amounts of the toxicants nitrite, sulfite and butyric acid (Table 4). Formic acid becomes toxic to the yeast when its concentration in the medium exceeds 0.25% (Table 4), whereas the toxicity level of the other two acids is in excess of 3.0% for the sum of the two. In baker's yeast fermentation, a maximum of about 40 g/L yeast dry matter end concentration in the fermentor can be reached. In industrial fermentations this will mean adding Dips to the mineral medium to an end dilution of 1:10 (Dips:medium). Since Dips is added in a fed-batch process, the concentration of toxic acids will increase gradually to reach about 0.3% for formic acid and about 0.7% for total acids at the end of fermentation (the amount present in Dips diluted 1:10). This end concentration of formic acid is within levels toxic to baker's yeast (Table 4). Fermentation experiments done in our laboratories confirmed this, where only 50-60% of the theoretical yields were obtained when pure Dips was used

as substrate (Al-Eid et al., 2009).

To try to remove the toxic compounds (the volatile fatty acids) from Dips, and hence increase yield, the treatment described in materials and methods was applied. This treatment did not bring about the desirable effect as can be seen from Table 4. This can be due to the fact that our treatment was performed in a simple pot, while the treatment of molasses is done in equipments specially designed for this purpose with heating up to 140 °C under pressure followed by release of pressure and injection of air, which can result in a more effective evaporation of the volatile toxic compounds (Bronn, 1990). Such equipment is not available to us now, but can be regarded as a possible solution in plants for commercial production. Another solution for this toxicity problem can be reached by the addition of molasses to Dips at 1:1 ratio. The dilution effect will bring the end concentration of formic acid to the half, i.e., 0.15%, which is below the toxicity level shown above. This solution was tried in fermentation experiments done in our laboratories and gave yields up to 90% of the theory, which were comparable to yields from pure molasses (Al Eid et al., 2009). This means that Dips can be used at up to 50% of the production substrate, which is a reasonable amount of utilization.

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Tables

Table 1. Dips contents from nutrients needed by baker's yeast.

Chemicals	Concentration in Dips	Contents in baker's yeast*
Phosphorus (g/kg)	1.13	14.10
Potassium (g/kg)	14.88	20
Magnesium (g/kg)	0.79	2
Total sugars (%)	80	30
Fructose	41	
Glucose	38	
sucrose	1	
Crude protein (%)	2	50
Pantothenic acid (ppm)	240	150
Biotin (ppm)	2.73	0.30
m-Inositol (ppm)	0	2000

* Bronn (1990).

Table 2. Composition of mineral media required for 400 g yeast dry matter.

Chemicals	Amount (g)		
	100% molasses	100% Dips	1:1 Dips/molasses
$(NH_4)_2SO_4$	61	60	60
$(NH_4)_2HPO_4$	54	53	52
Pantothenic acid	0.025	0	0
m-Inositol	0	0.8	0.4

Table 3. Comparing Dips and molasses as substrates for baker's yeast production.

Nutrient	Dips	Beet molasses*	Cane molasses*
Sugars (%)	80	50	50
Nitrogen (%)	0.13	0.5	0.1
Phosphorus (%)	0.11	0.03	0.09
Potassium (%)	1.5	3.0	3.0
Magnesium (%)	0.08	0.01	0.3
Biotin (ppm)	2.73	0.05	2.0
Pantothenic acid (ppm)	240	80	25
m-Inositol (ppm)	0	6500	2000

* Bronn (1990).

Table 4. Contents of Dips from chemicals toxic to baker's yeast.

Chemical	Toxicity level (%) in yeast substrate*		Concentration (%) in Dips	
	Tolerable	Toxic	Untreated	Treated
Nitrite	<0.001	Up to 0.05	0	0
Sulfite	<0.01	>0.15	0	0
Formic acid	<0.1	>0.25	3.06	2.81
Acetic acid			2.38	2.38
Propionic acid	<1.0	Up to 3.0	0.68	0.51
Butyric acid			0	0

* Bronn (1990).

Performance of Baker's Yeast Produced Using Date Syrup Substrate on Arabic Bread Quality

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Keywords: date syrup, molasses, baker's yeast, fermentation, Arabic bread, gas production power

Abstract

Baker's yeast was produced from three selected baker's yeast strains using date syrup as a substrate at low and high flow rate compared to those produced using molasses substrates. Performance of the produced baker's yeasts on Arabic bread quality was investigated. Baking tests showed a positive relationship between total Arabic bread quality and yeast gassing power. This relationship could be used as a general reference to estimate the quality of such yeasts. The Arabic bread made with Hollandia yeast produced using date syrup at low flow rate exhibited the highest overall total quality bread score. Moreover, NCYC 1530 baker's yeasts produced using date syrup at high flow rate or 1:1 molasses to date syrup at low flow rate, significantly produced high quality Arabic bread. Results also indicated that yeasts produced from the date syrup gave baking results comparable to yeasts produced from molasses. There were insignificant differences in gassing power between yeasts produced from date syrup and yeasts produced from molasses. So, it could be concluded that excellent quality baker's yeast could be produced using date syrup substrate.

INTRODUCTION

Dates contain high amounts of easily metabolizable sugars in the form of glucose and fructose therefore; they can be used as a substrate for microbial fermentations to produce a variety of commercial products such as baker's yeast, fodder yeast, ethanol, citric acid, vinegar. In these processes dates will serve mainly as carbon and energy sources for the microorganisms, in addition to other nutrients such as minerals and vitamins. In this respect date can compare very well with other conventional substrates used in industrial fermentations such as molasses. All baker's yeasts produced and used commercially in the world now, are strains of the species *Saccharomyces cerevisiae* that belongs to the fungal family *Saccharomycetaceae*. It is a unicellular, eukaryotic microorganism, usually diploid, reproduces vegetatively by budding and sexually by ascospore formation (Barnett et al., 2000). The role of the yeast in bread making is the raising of the dough to produce the characteristic loaf preferred by consumers. Dough rising occurs as a result of the gases produced by the yeast as it grows in the dough. During growth, the yeast metabolizes the sugars in the dough with the help of a special enzyme system and produce alcohol and CO_2. The leavening power of the yeast depends on its activity and viability; hence the yeast used must be fully active with a high viable cell count. Furthermore, the leavening power of any yeast strain depends on its genetic make up and on the production process and also on the storage conditions before use (Pyler, 1988).

Baker's yeast as a commercial product appears in several forms that can be grouped into two main types, compressed yeast, also called fresh yeast, and dried yeast. Compressed yeast represents the traditional form of baker's yeast. It is prepared by centrifugation and filtration after the completing of the fermentation process. It has a dry matter content of about 27-34% (w/w) and a protein content of about 42-56% (on dry-weight basis). It is ready for immediate use and, if it is handled properly, it should give

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good baking results in all types of dough systems. However, this type of yeast is perishable and should be stored at low temperatures, preferably between 0 and 4°C. At such temperatures, a shelf life of 3-4 weeks is possible with only a slight decrease in leavening capacity. At higher storage temperatures, keeping quality decreases progressively (Pyler, 1988).

Few investigations about the production of baker's yeast from date extracts have been conducted (Bassat, 1971; Mudhaffer, 1978; Al Obaidi et al., 1985, 1987). Comparisons were made between date extract and molasses, which is the traditional substrate for baker's yeast production. Positive findings were reported and claims were made that there are no technological constraints in using date extract for baker's yeast production. Nancib et al. (1997) used date wastes in the production of baker's yeast from strains of *Saccharomyces cerevisiae*. Date extract as carbon and energy source for the propagation of baker's yeast on pilot plant scale in comparison with molasses was investigated by Al Obaidi et al. (1986). The results showed that higher productivity of baker's yeast was observed when date extract was used. It was concluded from their study that date extract holds promise as a source of carbon and energy for the production of baker's yeast, although the average yields they reached were 47% only. None of these authors discussed the Crabtree Effect as a major technological problem encountered with baker's yeast propagation.

In a previous work the authors of this paper carried out a comparative study between nutrients contents of date syrup (Dips) and molasses as substrates for baker's yeast production. Results indicated that date syrup compares very well with molasses in its nutrients content and could be successfully used as a substrate for baker's yeast production (Al-Jasass et al., 2009).

Arabic bread has a round shape and forms pockets because of the high baking temperatures that make the dry exterior skin to set and carbon dioxide and water vapor to expand. These gasses disperse till the pressure is sufficient enough to force separation of the lower and upper layers, a phenomenon referred to as pocket formation (Quail et al., 1993). Qarooni et al. (1987) reported that white flour produced from hard wheat with intermediate strength and protein content of 10-12% gave high quality Arabic bread. The present study aims to evaluate the effect of using baker's yeast produced using date syrup as a substrate on quality characteristics of Arabic bread.

MATERIALS AND METHODS

Baker's Yeasts

The baker's yeasts used in this study were Saf-instant active dry baker's yeast (S. I. Lesaffre 59703 Marcq, France), Hollandia instant-active dry yeast (GB Ingredients, Mijlweg 77, Dordrecht, Holland), and strain from Specialized Laboratory: A *Saccharomyces cerevisiae* strain NCYC 1530 (laboratories of the National Center for Yeast Collection, Institute of Food Research, Norwich, Great Britain). The strain NCYC 1530 was provided in a freeze-dried form and was kept in its original form until use. These yeasts were produced using three substrates being (molasses, date syrup, and 1:1 molasses to date syrup) at low and high flow rates as described by Al-Eid et al. (2009), and were used in the Arabic bread preparation. The sugars content was 80 and 50% for date syrup and beet molasses respectively. The nitrogen content was 0.13 and 0.5% for date syrup and beet molasses respectively.

Flour

The flour used in the experiments was commercial local flour from hard wheat ('Yecora Rojo' variety), extraction rate of 80% and production year 2006, obtained in 10-kg paper packs (Grain Silos and Flour Mills Organization, Dammam, Saudi Arabia). The flour had 13.4% protein and 0.65% ash.

Flour Tests

1. Falling Number Test. The falling number was determined according to the method that is standardized by international bodies (Perten Instruments group, 2009), using the Perten instrument model 1700, Hagberg, Sweden. Flour (6.9 g) was weighed based on 14% moisture content, transferred into the viscometer-tube tipped to 45 angel, 25 ml distilled water added, the tube was shaken 10 times to obtain a uniform suspension of flour and water, and placed with a stirrer in the boiling water bath. The stirring apparatus was started immediately. Heating (about 100°C), causes starch granules to swell and the viscosity of the suspension to increase. After stirring the sample for 60 s, the plunger was dropped for free fall. The rate and extent to which the viscosity of the starch suspension was reduced indicated the level of alpha amylase present. The optimum falling number is obtained in the reading between 200 and 300. This indicates optimal amylase activity, and due to this the wheat bread crumb is likely to be good.

2. Farinograph Test. A farinograph measures and records the resistance of dough to mixing. It is used to evaluate the water absorption capacity of the flour to determine the stability of dough during mixing. Measurement was done according to the AACC (1995) approved method 54-21 with the small (50 g) mixing bowl of a Brabender farinograph (C.W. Brabender Instruments, Inc. South Hackensack, N.J, USA). After one minute dry mixing, sufficient water was added within 25 s, till maximum resistance was centered on the 500 Brabender Unit line. When the dough began to form, the sides of the bowl were scraped down with a plastic scraper, and the machine was permitted to run until an adequate curve was available (i.e., absorption, slightly beyond peak; stability, until top of curve recorded 500 BU line after peak; 12 min beyond the peak). The test was run three times, and then the absorption was calculated on a 14% moisture basis

3. Wet Gluten and Gluten Index. Gluten indices of flour samples were determined by the AACC (1995) approved method 38-12 using the Glutomatic 2200 (Perten Instrument AB, Stockholm, Sweden). Wet gluten is washed from the flour sample, centrifuged through a sieve and the weight of wet gluten forced through the sieve and that of the total wet gluten were measured. The total wet gluten was expressed as percent of sample, and the gluten index was expressed as percentage of wet gluten remaining on the sieve after centrifuging.

Arabic Bread Baking

Baking was done according to Qarooni et al. (1987). A modified bread formula was chosen for this experiment. Dry ingredients: 700 g flour, 7 g salt and 7 g yeast on dry weight basis (18 different bread treatments were prepared using the differently produced baker's yeast treatments: molasses, date syrup, and 1:1 molasses to date syrup) at low and high flow rates as described by Al-Eid et al. (2009) and were mixed at low speed for 1 min using a Hobart mixer Model A-120 (The Hobart Manufacturing Company, Tory, Ohio), then water was added (30°C) and mixing continued for 2 min at low speed and then for 4 min at medium speed. The optimum amount of water was determined using the formula of Qarooni (1989) and Qarooni et al. (1993), with some modifications based on experimental experience (baking absorption (%)=20+0.596 multiplied by the water absorption capacity determined by the farinograph test described above). The dough was then left to ferment in bulk for 1h at 30°C and 85% relative humidity (rh), then it was divided into pieces of 80 g, rounded by hand, covered with a cloth and allowed to relax for 15 min in the same fermentation cabinet. These dough pieces were then flattened by hand and cross sheeted in a machine (0.8 mm thickness). All sheeted doughs were put in a wooden board and transferred into the proofing cabinet for 30 min at 30°C and 85% rh. The proofed pieces were put on a pre-heated solid aluminum tray and baked at 425°C for 100 s in a bench top furnace (Muffle-Thermolyne 6000 Series). Loaves were cooled for 15 min and wrapped in plastic bags.

Yeasts Gassing Power

Samples of dough prepared as described above were used for the estimation of

yeast activity by measuring the gas production ability of the yeast. Since no fermentograph was available in our laboratory, measuring cylinders were used for assessing gas production ability. Twenty grams of dough was carefully placed in a 100-ml glass measuring cylinder, which was then incubated at 30°C and 90% relative humidity in a National MFG incubator (National MFG. Co., Lincoln, Nebraska). The increase in dough volume due to CO_2 production was measured every 30 min for 2h (Hamad and Al Eid, 2005).

Sensory Evaluation of Arabic Bread

A scoring system for quality parameters based on a numerical scale reported by Qarooni et al. (1987) was used. The scores sheet was assigned according to consumer preference in the Middle Eastern countries. At least six loaves of bread (14-16 cm diameter) were baked for each treatment, and were evaluated on the first day, then kept overnight at room temperature in plastic bags for evaluation on the second day. On the first day, a total of 47 points were given to external quality factors and 53 points to internal factors, whereas a total of 50 points were given for the second day evaluations. In all cases, the higher the score the better the individual quality of the breads (Qarooni et al., 1987).

Sensory assessment for bread quality was performed by 13 volunteer panelists chosen among students and technicians from the King Faisal University, Saudi Arabia. They were nonsmokers aged 19-38 years, semi-trained and received instructions regarding the evaluation procedures both verbally and written. The panelists performed the tests at self-determined pace with no time limit, though the evaluation sessions were intended to last 15-30 min. To minimize adaptation, panelists were instructed to take breaks of 2-3 min as they desired and the evaluation was associated with the control. Evaluation was conducted after 1h of baking and on the next day.

Experimental Design and Statistical Analysis

A 3×3×2 factorial experimental design was employed with three types of substrate, three yeast strains, and two feeding flow rates as variables. A randomized block design was chosen to run the experiments. Analysis of variance (Steel and Torrie, 1980) of the data collected during the course of each trial was performed. Analysis of Variance (ANOVA) was performed to estimate the interaction effect among independent variables using SAS software (Ver. 6.02). Least Significant Differences (LSD) ($P>0.05$) between treatment means were determined using Fisher's test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Flour Protein Content

The average protein content for flour (80% extraction) used in this study was 13.4%. The results obtained were similar with results reported by Quail (1990) who found that high extraction flours may have protein contents slightly higher than the corresponding straight run flour. Qarooni et al. (1988) found that the optimum flour protein for Arabic bread was 10-12% whilst Quail et al. (1991) found that flour with a protein content of 9-12% was suitable. So, this protein content is slightly higher than those recommended by Qarooni et al. (1988) and Quail et al. (1991) and may result in a decrease in dough strength and elasticity.

Farinograph

It was found that farinograph water absorption was 66.6% for flour used in this study. These results are in agreement with those reported by Quail et al. (1991) who, reported that water absorption should be high for flours used for Arabic bread production with farinograph values ranging from 58 to 65%. Also, Qarooni (1988) defined preferred water absorption greater than 60% suitable for Arabic bread. So, the farinograph test is a useful complement to the baking test because it pinpoints the causes of poor baking

performance of flours (Bloksma and Bushuk, 1988).

Wet Gluten and Gluten Index

All reported data are averages of three replicates. Gluten of tested flour indicated high wet gluten weight being 29.71 g. On the other hand, the gluten index value and dry gluten content were 99.36% and 11.85 g, respectively. Duska et al. (2001) found that the gluten index in flours made from the most important Croatian wheat cultivars varied from 55.92 to 99.6%. Pyler (1988) reported that a more or less definite correlation also exists between the wet and dry gluten values, with those for wet gluten being slightly over three times higher than that for dry gluten. The ratio of wet to dry gluten was used as an index of protein quality in the belief that the water-holding capacity represents a significant quality attribute. The quantity and quality of gluten are considered the most important quality parameters of wheat flour responsible for physical dough properties (Duska et al., 2001).

Falling Number

The falling number is a method of determining the relative level of alpha amylase activity. The falling number of the experimental flour was 447 s. However, Brain (2005) indicated that a falling number value of 350 s or longer indicate low enzyme activity and very sound wheat. As the amount of enzyme activity increases, the falling number decreases. Values below 200 s indicate a high level of enzyme activity.

Yeast Activity (Gas Production Power)

Samples of the prepared bread dough used for Arabic bread making were also used for estimation of yeast activity through gas production ability. Values of gas production power (ml/20 g dough) of the different experimental yeast treatments are presented in Table 1. It could be noted that there were significant differences due to the substrate used in yeast propagation and also for the flow rate used. However, there was insignificant effect to yeast strain in this respect. The highest significant effect on the gas production power was noticed for the flow rate. Usually, the baker's yeast produced using the low flow rate showed significantly higher gas production power than those produced following the high flow rate system.

Concerning the substrate used in the yeast production, it could be noticed there are insignificant differences for the gas production power between yeasts produced using either molasses or date syrup substrates. However, the highest gas production power was noticed for yeasts produced using 1:1 molasses to date syrup substrate at the low flow rate system after 60, 90 and 120 min of incubation. These conditions of growth give biochemical properties of the yeast a special emphasis, and demand specific measures to ensure high quality and efficient dough aeration (Stear, 1990). Joshi et al. (2005) used apple pomace in comparison to molasses under fed batch cultivation for baker's yeast production. There was no appreciable difference in the dough raising capacity of the produced yeast compared to the commercial yeast that was evident. These findings demonstrate that apple pomace is a comparable energy source for the production of baker's yeast. The technological role of yeast in wheat-flour dough is a strong alcoholic fermentation with extensive carbon dioxide liberation. The gassing power of pressed yeast depends on the zymase enzyme-complex of the yeast cells, and available fermentable carbohydrates (Stear, 1990). The differences in the gassing power in the produced yeasts used in this work might be due to the various maltase and zymase activities of these yeasts.

Arabic Bread Quality

The effect of the three selected baker's yeast strains produced from date syrup and molasses substrates under low and high flow rates on the quality of Arabic bread was investigated. Analysis of Variance (ANOVA) was performed to estimate the interaction effect among independent variables. Separation of means among all external and internal

quality parameters were analyzed using least significant parameters, Fisher's (LSD) test.

Crust Smoothness and Shape

The ANOVA analysis showing the effect of baker's yeasts from different fermentation treatments on Arabic bread quality can be observed in (Table 2). There was strong interaction (Yeast×Substrate×Flow rate) effect on crust smoothness ($p \leq 0.05$) and shape ($p \leq 0.01$) values of Arabic bread. Bread made with yeasts from date syrup showed no significant decrease in crust smoothness and shape among all yeast strains compared to those produced using molasses. Arabic bread made with yeasts and produced under the following parameters: 1:1 molasses to date syrup, Hollandia yeast, and high flow rate (Treatment #12) showed the highest score for bread shape as compared with other treatments (Table 3). All breads showed a marked significant variation in crust smoothness.

Crust Color, Cracks and Blisters

Arabic bread showed a marked variation in crust color, cracks and blisters among all treatments. There is a strong significant interaction effect (Yeast×Substrate×Flow rate) on crust color ($p \leq 0.05$) as well as blister appearance and crakes ($p \leq 0.01$) of the Arabic bread as shown in Table 2. It could also be noted from results of Table 3 that there were insignificant difference in crust color, cracks and blisters in case of using date syrup baker's yeast as compared with those of molasses, but yeast produced from 1:1 date syrup to molasses showed the highest score for these bread properties.

Ability to Roll and Fold

The ability of Arabic bread to roll and fold was evaluated for the first and second day after baking. There was a strong interaction effect (Yeast×Substrate×Flow rate) on the roll and fold features of Arabic bread on the first day and on the second day ($P \leq 0.01$) as can be seen from Table 2. Arabic bread made with yeast produced using date syrup as a substrate, low flow rate and Hollandia yeast (Treatment #9) scored the highest value on the ability to roll and fold on the first as well as on the second day (Table 3).

Separation and Evenness of Layers

There was insignificant interaction effect due to the (Yeast×Substrate×Flow rate) on the quality of separation of layers. However, there was a strong interaction effect ($P \leq 0.01$) due to the (Substrate×Flow rate) on the quality of separation of layers (Table 2). There was a significant interaction effect ($P \leq 0.01$) due to the (Yeast×Substrate×Flow rate) on the evenness of layers. Arabic breads showed insignificant variation in the scores of both separation and evenness of layers among all treatments (Table 3) which indicate that use of date syrup baker's yeast resulted in good quality Arabic bread.

Grain Appearance, Grain Uniformity, Crumb Texture and Crumb Color

There was insignificant interaction effect due to the yeast, substrate, flow rate as well as their interactions on grain appearance, grain uniformity, crumb texture and crumb color. However, the only significant interaction effect ($P \leq 0.01$) was due to the (Yeast×Substrate×Flow rate) on the grain uniformity, and effect ($P \leq 0.05$) due to (Yeast×Flow rate) on crumb color. There was strong effect ($P \leq 0.01$) due to substrate on crumb color (Table 2). All Arabic breads showed no significant differences in values of grain appearance, grain uniformity and crumb texture among all treatments (Table 3). Bread made with yeasts produced from date syrup only showed the highest crumb color scores (Table 3).

Tearing Quality

The ability of Arabic bread to tear in an acceptable manner was evaluated for the first and the second day. The interaction effect due to (Yeast×Substrate×Flow rate) on the tearing feature of Arabic white bread was significant on the first day and on the second

day (P≤0.1) (Table 2). Tearing quality of Arabic bread made with yeasts from Saf-Instant and The NCYC 1530 strain showed insignificant variations in tearing quality in the first day as could be seen from (Table 3). However, there was a marked significant variation in tearing quality of Arabic bread in the second day among all treatments (Table 2).

Total Score

There was a significant interaction effect due to the (Yeast×Substrate×Flow rate) on the total score of the Arabic bread (P≤0.01) as can be seen in Table 2. Ferrari et al. (2001) produced baker's yeast production from molasses/cheese whey mixtures, molasses substitution of 46%, in terms of sugar fed to the bioreactor, was reached and no significant differences in baking quality was observed. One out of 18 treatments exhibited the highest overall total quality bread score. The ninth treatment was the Arabic bread made with yeast produced using date syrup, low flow rate and Hollandia yeast as shown in Table 3. Two treatments (16 and 17) also exhibited a high total quality bread score (Table 3). One of these two treatments were the Arabic breads made with yeast produced using date syrup, high flow rate and NCYC 1530 yeast strain. The second was the Arabic bread with yeast made from 1:1 molasses to date syrup, NCYC 1530 yeast strain, and low flow rate. Pyler (1988) reported that bread doughs prepared only from flour, water, yeast and salt (such as Arabic bread) will initially contain only about 0.5% of glucose and fructose derived from the flour. This is adequate to start fermentation and to activate the yeast's adaptive malto-zymase system that is responsible for maltose fermentation. Different yeast strains have been shown to vary in their maltase activity and may also exhibit variable maltase activity under different dough conditions. Yeast itself brings the same changes in the bread dough in the course of fermentation, such as depletion of fermentable substances, accumulation of products in the form of carbon dioxide, alcohols, acids and esters, modification of pH conditions, and softening or mellowing of the gluten character. In this present study, all of these factors may have contributed to the quality differences among baker's yeast produced in this study.

CONCLUSIONS

Baker's yeasts produced using the date syrup substrate gave baking results and gas production power comparable to those of yeasts produced from molasses, indicating that using date syrup as substrate for beaker's yeast production results in excellent quality yeasts to be used in the baking industry.

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Tables

Table 1. Gas production power (ml/20 g dough) for baker's yeasts (LSD Test*).

Run	Substrate	Flow rate	Incubation time (min)				
			Zero	30	60	90	120
Saf-Instant							
1	Molasses	Low	19^{b}	43^{a}	46^{a}	47^{b}	47^{d}
2	Molasses	High	18^{c}	37^{b}	42^{d}	44^{e}	46^{e}
3	Date syrup	Low	19^{b}	37^{b}	44^{b}	47^{b}	47^{d}
4	Date syrup	High	18^{c}	35^{d}	43^{c}	46^{c}	46^{e}
5	1:1	Low	21^{a}	35^{d}	42^{d}	46^{c}	50^{a}
6	1:1	High	17^{d}	35^{d}	42^{d}	45^{d}	46^{e}
Hollandia							
7	Molasses	Low	18^{c}	34^{e}	44^{b}	46^{c}	49^{b}
8	Molasses	High	18^{c}	22^{m}	29^{i}	37^{i}	42^{i}
9	Date syrup	Low	18^{c}	31^{f}	41^{e}	45^{d}	48^{c}
10	Date syrup	High	17^{d}	26^{i}	36^{f}	44^{e}	46^{e}
11	1:1	Low	17^{d}	29^{j}	33^{g}	45^{d}	46^{e}
12	1:1	High	17^{d}	24^{k}	33^{g}	41^{g}	44^{g}
Strain NCYC 1530							
13	Molasses	Low	18^{c}	31^{f}	42^{d}	44^{e}	45^{f}
14	Molasses	High	18^{c}	23^{l}	31^{h}	36^{j}	42^{i}
15	Date syrup	Low	19^{b}	25^{j}	42^{d}	43^{f}	45^{f}
16	Date syrup	High	18^{c}	34^{e}	43^{c}	44^{e}	46^{e}
17	1:1	Low	18^{c}	27^{h}	42^{d}	43^{f}	45^{f}
18	1:1	High	18^{c}	23^{l}	31^{h}	39^{h}	43^{h}
Control*			18^{c}	36^{c}	46^{a}	49^{a}	50^{a}

Control = Hollandia Yeast.

***** Within each column, means with the same letter are not significantly different.

Table 2. F-Factors (Analysis of Variance) of Arabic bread.

Quality attribute	Yeast	Substrate	Flow rate	Yeast×substrate	Yeast×flow rate	Substrate×flow rate	Yeast×Substrate×Flow rate
Crust smoothness	19.1***	7**	51**	4.8*	16**	NS	4.6**
Shape	4.2*	11.2**	NS	8***	37.2***	7.5**	99***
Crust color	6.3**	NS	NS	14.75***	6.8**	NS	6.2**
Cracks	7.2**	9.5**	21.8**	2.9*	22.3***	NS	2.3***
Blisters	34***	8**	NS	4.8*	28.1***	NS	10.4***
Roll & fold (1)	NS	8**	29.7**	NS	7.5**	NS	7***
Separation of layers	NS	13**	NS	26***	NS	45***	NS
Evenness of Layer	NS	NS	NS	3.9**	NS	NS	11.7***
Grain appearance	NS	NS	NS	NS	NS	NS	NS
Grain uniformity	NS	NS	NS	NS	NS	NS	3.3*
Crumb texture	NS	NS	NS	NS	NS	NS	NS
Tearing quality (1)	5.4*	NS	30.2**	NS	NS	NS	3.6*
Crumb color	18***	83.1***	NS	NS	7.1**	NS	NS
Roll & fold (2)	NS	99***	402***	19.9***	25.***	NS	32***
Tearing quality (2)	133**	18.6***	NS	NS	26.1***	24.5***	999***
Total	8**	79***	29**	8.5***	999***	NS	999***

* Significant at $p \leq 0.1$.
** Significant at $p \leq 0.05$.
*** Significant at $p \leq 0.01$.
NS Not significant.

Table 3. Quality characteristic of Arabic bread (LSD Test*).

Treatment	Substrate	Flow rate	Crust smoothness (5)	Shape (8)	Crust color (8)	Cracks (8)	Blisters (8)	Rolling & folding (1) (10)	Separation of layers (15)	Evenness of layers (5)	Grain appearance (5)	Grain uniformity (5)	Crumb texture (8)	Tearing quality (1) (10)	Crumb color (5)	Rolling & folding (2) (30)	Tearing quality (2) (20)	Total (150)
Saf-Instant																		
1	Molasses	Low	4.5^{c}	6^{d}	7.8^{ab}	6.8^{b}	8^{b}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.8^{ab}	13.3^{ef}	9.3^{c}	115.9^{hi}
2	Molasses	High	4.7^{c}	7^{b}	7.7^{abc}	6.9^{ab}	8^{b}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	$4.^{6c}$	11.3^{gh}	9.6^{c}	115.1^{i}
3	Date syrup	Low	5^{a}	7^{b}	8^{a}	6.9^{ab}	8^{b}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	5^{a}	13.9^{de}	9.8^{bc}	119.8^{cde}
4	Date syrup	High	4.8^{bc}	7^{b}	8^{a}	6.9^{ab}	6.9^{e}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	5^{a}	13.3^{ef}	9.6^{c}	116.5^{fgh}
5	1:1	Low	5^{a}	7^{b}	8^{a}	6.9^{ab}	6^{b}	10^{a}	14.8^{b}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	5^{a}	13.5^{de}	9.3^{c}	117.3^{efg}
6	1:1	High	4.9^{ab}	7^{b}	7.8^{ab}	6.9^{ab}	7.6^{cd}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.8^{ab}	11.6^{efg}	9.5^{c}	115.4^{i}
Holendia																		
7	Molasses	Low	5a	7^{b}	8^{a}	6.9^{ab}	7.8^{cb}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	7.7^{e}	4.5^{d}	9.3^{i}	$7d^{e}$	109.9^{ijk}
8	Molasses	High	4.5^{c}	6.7^{b}	7.8^{ab}	6.9^{ab}	8^{b}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.7^{c}	9.3^{i}	6.2^{e}	108.3^{k}
9	Date syrup	Low	5^{a}	7^{b}	7^{e}	6.9^{ab}	7.5^{d}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	7.9^{ab}	5^{a}	21.6^{a}	8^{d}	123^{a}
10	Date syrup	High	5^{a}	7^{b}	8^{a}	6.9^{ab}	8^{b}	9.8^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	7.9^{ab}	5^{a}	14^{de}	9.6^{c}	116.5^{cde}
11	1:1	Low	4.8^{bc}	7^{b}	7.7^{bc}	6.9^{ab}	7.8^{bc}	10^{a}	15^{ab}	4.8^{b}	5^{a}	5^{a}	7^{a}	7.8^{d}	4.8^{ab}	18^{b}	8^{d}	119^{bcd}
12	1:1	High	4.8^{bc}	7.9^{a}	7.9^{ab}	6.9^{ab}	8.3^{a}	8.8^{c}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	7.9^{c}	5^{a}	12.3^{efg}	9.3^{c}	116.1^{gh}
Strain																		
13	Molasses	Low	5^{a}	7^{b}	7.2^{de}	6.9^{ab}	8^{b}	9.8^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.6^{c}	13.3^{ef}	12.3^{a}	116.1^{def}
14	Molasses	High	5^{a}	6.6^{cb}	7.5^{cd}	6.4^{c}	8^{b}	9.3^{bc}	14.6^{c}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.5^{d}	10^{hi}	9^{c}	111^{j}
15	Date syrup	Low	5^{a}	7^{b}	8^{a}	6.9^{ab}	8^{b}	10^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	5^{a}	13.6^{de}	12.3^{a}	120^{bcd}
16	Date syrup	High	5^{a}	6.3^{cd}	7.9^{ab}	6.9^{ab}	8^{b}	9.6^{ab}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	5^{a}	16^{c}	12^{a}	121^{ab}
17	1:1	Low	5^{a}	7^{b}	7.8^{ab}	7^{a}	8^{b}	9.6^{ab}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	8^{a}	4.8^{ab}	14^{de}	12.6^{a}	121^{ab}
18	1:1	High	5^{a}	6.1^{d}	7.7^{bc}	6.5^{c}	8^{b}	9.6^{ab}	15^{ab}	4.8^{b}	5^{a}	5^{a}	7^{a}	8^{a}	4.8^{ab}	15^{c}	10.6^{b}	118.7^{cde}
Control			4.8^{bc}	6.9^{b}	8^{a}	7^{a}	7.8^{bc}	9.8^{a}	15^{ab}	5^{a}	5^{a}	5^{a}	7^{a}	7.8^{ab}	5^{a}	13^{f}	9^{c}	115.1^{i}

Control=Hollandia Yeast

* Within each column, means with the same letter are not significantly different.

Effects of Plant Growth Regulators on Quantitative and Qualitative Traits of 'Shahani' Date (*Phoenix dactylifera*) Fruits

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Keywords: 'Shahani' date palm, plant growth regulator

Abstract

In order to evaluate the effects of plant growth regulators on quantity and quality traits of 'Shahani' date fruits, this study was performed in two successive years as factorial in RCBD arrangement with 4 replications. Clusters of tested palms were pollinated with distinct and equal pollen and then treated with different concentrations of BA, GA_3, NAA and 2,4-D, alone or in combination, in two stages: hababook and keimeri at ripening stage traits were measured including: ripening percentage, TSS, pH, water percentage, fruit and pit weight, length and diameter. Results showed that in treatments having 2,4-D the fruit size and in treatments having GA_3 and BA the core size becomes large. TSS in the control treatment was more than other treatments. Treatments having auxin and GA_3 showed delayed ripening in comparison with other treatments. Generally, treatments having BA with low concentrations of auxin and GA_3 were suitable for improvement of quantity and quality traits of 'Shahani' date fruits.

INTRODUCTION

Undoubtedly date palm is the oldest plant that there was in the world and has been the original source of earning a living of mankind. The origin of date palm is Iraq and the western and southern regions of Iran. At present, more than 50% of dates in the world world are produced in Khouzestan plain, Mesopotamia, the beaches of Arvand and Karoon rivers, in other words in Iran and Iraq (Rohani, 1988). The main date palm culture regions in Iran are Khouzestan, Kerman, Hormozgan, Fars, Boushehr, Sistan and Balouchestan, Esfahan provinces and a few in other provinces (Agriculture Ministry, 1997). Whereas the world production of date is increasing and Iran is among the main date producers in world, each type of research can be effective in increasing of production and quality of date fruit and as the most economic and non-oil crop for export. Plant growth regulators are used to enhance qualitative and quantitative traits of different fruits such as date fruit. The effects of plant growth regulators depend on application time and method as well as plant species. These materials are used in date fruit in order to enhance weight and volume of fruits, chemical thinning, prevention of fruit wrinkle, post-, even and early ripening of fruit and color enhancement. In this relation auxins, cytokinins, gibberilins and ethylene have much use (Aboutalebi and Behrouznam, 2006). There is much auxin in the initial stage of fruit set in date fruit. This hormone causes cell division as well as cell growth. Sharples and Hilgeman (1950) reported that application of auxins in keimeri stage and early khalal stage has no effects on growth and development as well as fruit maturity. Gerogea and Hilgeman (1950) showed that application of 20 and 30 mg/L 2,4-D and 2,4,5-T on 'Sayer' cultivar caused fruit enlargement. Shafaat and Shabana (1980) reported that application of NAA 15-16 weeks after pollination on 'Zahidi' date fruit caused enhancement of fruit size, weight and volume and pulp/stone ratio and fruit moisture but was not effective on total soluble solids. Ripening time delayed and maturity delay in treated fruits in the beginning and the end of khalal stage was the lowest. Gibberillins have an effective role in cell enlargement and protein synthesis. This hormone is produced in seed and its artificial application has been increased quantitatively and qualitatively on different fruits. El-Nabawy et al. (1977) reported that GA_3 application on 'Samani' cultivar increased fruit size but it decreased

fruit color. Together application of GA_3 and Ethephon increased TSS. Moujheith et al. (1979) explained that together application of GA_3 and Ethephon could not increase fruit size and quality compared to GA_3 alone but it increased TSS noticeably. Cytokinins are among hormones that simulate cell division and cell growth and it also increases protein synthesis and by this way it delays tissue senility. Hassaballa et al. (1984) reported that non-pollinated clusters in 'Zaghloul' date cultivar that was treated with BA (20 mg/L) in opening spath time and 2,4-D (100 mg/L) in early June and early July, have higher weight than pollinated clusters. They also reported that sugar content and TSS in treated fruits was lower than in control fruits. Hodeiri et al. (1998) reported that application of 10 mg/L 6-phurphuril amino purin + GA_3 on pollinated clusters of three date cultivars ('Simbebel', 'Talis' and 'Edvi') significantly increased fruit weight and length and produced seedless fruits.

The Jahrom township has a proper situation in date culture regions in Iran. In this township, 'Shahani' date cultivar has the highest culture surface and date production. Small fruit size in 'Shahani' date cultivar is the main problem in crop marketing. The effect of plant growth regulators in fruit size enhancement has been demonstrated in many crops. Whereas physiological characteristics of each plant and environmental conditions of culture region can be effective in final results, in this study inspired by the results of other researchers, the effects of plant growth regulators have been evaluated on quantitative and qualitative traits of 'Shahani' date cultivar.

MATERIALS AND METHODS

In order to evaluate the effects of plant growth regulators on quantitative and qualitative traits of 'Shahani' date cultivar, this study was performed in two successive years as factorial in RCBD arrangement with 4 replications. Clusters of tested palms were pollinated with distinct and equal pollen and then treated with different concentrations of BA (40 and 80 mg/L), GA_3 (25 and 50 mg/L), NAA (30 and 60 mg/L) and 2,4-D (50 and 100 mg/L), alone or in combination and water as control, in two stages: hababook and keimeri (6 and 10 weeks after pollination, respectively). At ripening stage traits were measured including: ripening percentage, TSS, pH, water percentage, fruit and stone weight, length and diameter. For measurement of weight was done by digital weighing machine (0.001) and length and diameter by caliper. For measurement of TSS 9 g was mixed fruit pulp with 45 ml distilled water and after 16 h TSS was measured by refractometer. In the above solution pH was measured by digital pH meter. To measurement of fruit water percent, 10 g fruit pulp was placed in 75°C for 48 h and then weighed. Obtained results were analyzed by MSTAT-C software and means were compared by Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

The Effect of Year, Treatment and Year and Treatment Interaction on Qualitative Traits

The results of variance analysis in relation to the effect of year, treatment and year-treatment interaction have significant effect at $P<0.01$ on measured qualitative traits such as fruit moisture percent, pH, TSS and fruit ripening percent (Table 1).

The Effect of Year, Treatment and Year and Treatment Interaction on Quantitative Traits

The results of variance analysis relative to the effect of year, treatment and year-treatment interaction have significant effect at $P<0.01$ on measured quantitative traits but there was no significant different in relation to year effect on stone weight and length. The effect of year-treatment interaction was significant at $P<0.05$ on stone diameter (Table 1).

Comparison of the Results of Two Years

Obtained results of two years were not significant in some cases and in others have significant different at P<0.05 of Duncan's test (Table 2). In this relation, stone weight and length in both years were without significant difference and in the same statistical level. Fruit moisture percent, TSS and fruit ripening percent in the second year with significant difference at P<0.05 was higher than in the first year. pH, pulp/stone ratio, stone diameter and fruit length, diameter and weight in the first year were higher than in the second year (Table 2).

Comparison of the Effect of Treatment Type on Qualitative Traits in Two Years

With regard to the results of two years of experiment, it was distinguished that under the effect of treatment type, some traits were higher than the control and others were lower than the control. In this relation, treatment type had a significant effect on fruit moisture percent at P<0.01 DMRT and the highest fruit moisture percent (25.93%) was in 20 mg/L BA + 20 mg/L GA_3 + 20 mg/L NAA and the lowest (16.48%) in 30 mg/L NAA (Table 3). Against these results, Shafaat and Shabana (1980) observed the highest fruit moisture percent in usage of NAA.

The fruits of mixed treatment of BA + 2,4-D each one in 50 mg/L concentration have the highest pH (7.44) and the lowest pH (6.92) in the fruits of 60 mg/L NAA treatment (Table 3). The highest TSS (74.0) was in the fruits of the control treatment and the lowest (60.8) in the fruits of the mixed treatment of BA + 2,4-D each one in 50 mg/L concentration (Table 3). These results completely conform to the results of Hassaballa et al. (1984) because using BA and 2,4-D as compared to the control reduced TSS.

Some treatments accelerate fruit ripening and others delay it. The highest ripened fruits percent (90%) was in 20 mg/L GA_3 and the lowest (70%) in 60 mg/L NAA and 50 mg/L 2,4-D. There is no significant difference between these two treatments with control at P<0.05 DMRT (Table 3). Similar to these results are the ones obtained by Shafaat and Shabana (1980) that using of NAA delayed fruit ripening in 'Zahidi' date.

Comparison of the Effect of Treatment Type on Quantitative Traits in Two Years

In regard to the results of two years of experiment, it was distinguished that under the effect of treatment type, some traits were higher than the control and others were lower than the control. In this relation, treatment type has significant effect on fruit diameter at P<0.05 DMRT and the highest fruit diameter (21 mm) was in the fruits of 100 mg/L 2,4-D and the mixed treatment of BA + 2,4-D each one in 50 mg/L concentration and the lowest fruit diameter (18.6 mm) was in the control treatment fruits (Table 4). Gerogea and Hilgeman (1950) also obtained the highest fruit diameter using 2,4-D which conforms to these results. The mixed treatment fruits of 50 mg/L BA + 25 mg/L GA_3 have the highest weight of fruit (13.7 g) and the control treatment fruits have the lowest weight of fruit (8.19 g) (Table 4). These results conform to the results of Nabavi et al. (1984), Hodeiri et al. (1998) and Moujheith et al. (1979). The longest fruit (52 mm) was in mixed treatment fruits of 50 mg/L BA + 25 mg/L GA_3 and the shortest fruit (42.6 mm) was in the control treatment fruits (Table 4). Hodeiri et al. (1998) also obtained the highest length of fruit by using 6-phurphurilamino purin + GA_3.

The highest weight of stone (1.19 g) was in the mixed treatment fruits of 20 mg/L BA + 20 mg/L GA_3 + 30 mg/L 2,4-D and the lowest of that (0.74 g) was in 50 mg/L GA_3 (Table 4). The highest length of stone (30.6 mm) was in the mixed treatment fruits of 50 mg/L BA + 25 mg/L GA_3 and the lowest of that (25.6 mm) was in 60 mg/L NAA (Table 4). The thickest stone (7.85 mm) was in the mixed treatment fruits of 20 mg/L BA + 20 mg/L GA_3 + 30 mg/L 2,4-D and the narrowest stone (6.67 mm) was in control treatment fruits (Table 4). The highest pulp/stone ratio (13.96) was in 40 mg/L BA and the lowest of that was in the mixed treatment fruits of 20 mg/L BA + 20 mg/L GA_3 + 30 mg/L 2,4-D (Table 4).

GENERAL CONCLUSION

Based on the total results, the treatments containing BA with the low concentrations of auxin or GA_3 had been properly distinguished for improvement of the quantitative and qualitative characteristics of 'Shahani' date fruit.

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Tables

Table 1. Variance analysis of the effect of year, treatment and year-treatment interaction on qualitative and quantitative traits.

Trait		Treatment mean square	Error mean square	Fisher value	Coefficient of variation (%)
Fruit moisture (%)	year	7200.206	2.581	2789.00**	7.85
	treatment	60.985		23.60**	
	year*treatment	64.424		25.00**	
pH	year	0.565	0.011	53.40**	1.43
	treatment	0.125		11.80**	
	year*treatment	0.173		16.40**	
TSS (%)	year	3635.319	5.973	609.00**	3.75
	treatment	114.074		19.10**	
	year*treatment	93.542		15.70**	
Ripening (%)	year	51.875	6.871	7.55**	3.27
	treatment	263.070		38.30**	
	year*treatment	233.729		34.01**	
Stone weight (g)	year	0.036	0.014	2.50ns	12.48
	treatment	0.108		7.54**	
	year*treatment	0.053		3.68**	
Stone length (mm)	year	3.772	1.557	2.42ns	4.49
	treatment	10.486		6.73**	
	year*treatment	5.581		3.58**	
Stone diameter (mm)	year	1.92	0.168	11.4**	5.69
	treatment	0.656		3.90**	
	year*treatment	0.364		2.16*	
Fruit length (mm)	year	272.520	4.91	55.50**	4.75
	treatment	27.767		5.66**	
	year*treatment	36.684		7.47**	
Fruit diameter (mm)	year	59.506	0.858	69.40**	4.73
	treatment	3.841		4.48**	
	year*treatment	5.311		6.19**	
Fruit weight (g)	year	267.129	1.097	244**	9.48
	treatment	11.186		10.20**	
	year*treatment	12.552		11.40**	
Pulp/stone ratio	year	230.277	4.566	50.40**	18.81
	treatment	12.691		2.78**	
	year*treatment	14.806		3.24**	

ns, *, **, not significant, significant at $P<0.05$ and 0.01, respectively.

Table 2. Comparison of the results of two years in relation to evaluated traits.

Trait	Year	
	2006	2007
Fruit moisture (%)	11.20b	29.70a
pH	7.30a	7.14b
TSS (%)	58.63b	71.79a
Ripening (%)	13.02a	9.71b
Stone weight (g)	0.94a	0.98a
Stone length (mm)	27.6a	28.00a
Stone diameter (mm)	7.37a	7.06b
Fruit length (mm)	48.46a	44.86b
Fruit diameter (mm)	20.43a	18.75b
Fruit weight (g)	12.83a	9.27b
Pulp/stone ratio	79.29b	80.86a

Within each row, means followed by the same letter are not significantly different ($P<0.05$) using Duncan's multiple range tests.

Table 3. Comparison of the effect of treatment type on qualitative traits in two years of experiment.

Treatment	Trait			
	Fruit moisture (%)	pH	TSS (%)	Ripening (%)
Control	19.88ef	7.15e	74.0a	72.50f
BA 40[†]	20.50ef	7.31bcd	69.2b	85.17bc
BA 80	19.72ef	7.40ab	65.6c	84.83bc
GA_3 20	16.85gh	7.34abc	70.0b	90.00a
GA_3 50	17.00gh	7.23cde	71.6ab	80.00de
NAA 30	16.48h	7.15e	61.8de	72.50f
NAA 60	21.77de	6.92f	63.6cde	70.00f
2,4-D 50	17.23gh	6.98f	66.3c	70.00f
2,4-D 100	23.78bc	7.20de	61.2de	84.00c
$BA_{20}+GA_{20}+NAA_{20}$	25.93a	7.19de	64.2cd	87.50ab
$BA_{20}+GA_{20}+2,4\text{-}D_{30}$	18.75fg	7.22cde	61.5de	82.00cd
$BA_{50}+GA_{25}$	23.20cd	7.28bcde	61.6de	85.00bc
$BA_{50}+NAA_{30}$	25.60ab	7.23cde	61.6de	77.50e
$BA_{50}+2,4\text{-}D_{50}$	19.78ef	7.44a	60.8e	80.00de

Within each column, means followed by the same letter are not significantly different ($P<0.05$) using Duncan's multiple range tests, [†] The unit of treatments is mg/L.

Table 4. Comparison of the effect of treatment type on quantitative traits in two years of experiment.

Treatment	Trait						
	Fruit diameter (mm)	Fruit weight (g)	Fruit length (mm)	Stone weight (g)	Stone length (mm)	Stone diameter (mm)	Pulp/stone ratio
Control	18.6c	8.19g	42.6d	0.84def	26.2fg	6.67e	9.71c
BA 40[†]	19.4c	11.76bc	47.6bc	0.80ef	26.7efg	6.98cde	13.96a
BA 80	19.3c	11.10bcd	46.4bc	1.09ab	28.7bdc	7.73ab	9.74c
GA_3 20	19.0c	9.36fg	44.7cd	0.89cdef	27.8bcdef	7.05cde	12.00abc
GA_3 50	18.9c	10.24def	45.3bcd	0.74f	26.7efg	7.00cde	13.28ab
NAA 30	19.8bc	9.76ef	45.3bcd	0.99bcd	27.1defg	7.28bcd	9.53c
NAA 60	18.9c	10.79cde	45.2bcd	0.91cde	25.6g	7.25bcd	11.30abc
2,4-D 50	19.5c	10.87cde	47.3bc	0.87cdef	27.5cdef	7.05cde	12.04abc
2,4-D 100	21.0a	12.34b	47.6bc	1.09ab	29.3ab	7.43abc	10.95bc
$BA_{20}+GA_{20}+NAA_{20}$	19.8bc	11.93bc	47.2bc	1.00bc	27.7bcdef	7.28bcd	11.40abc
$BA_{20}+GA_{20}+2,4\text{-}D_{30}$	20.8ab	11.43bcd	46.0bc	1.19a	28.4bcd	7.85a	9.35c
$BA_{50}+GA_{25}$	19.7bc	13.70a	52.0a	1.12ab	30.6a	7.43abc	11.62abc
$BA_{50}+NAA_{30}$	18.8c	11.72bc	47.8b	1.02bc	28.9bc	7.23bcd	11.08abc
$BA_{50}+2,4\text{-}D_{50}$	21.0a	11.49bcd	48.0b	0.84def	27.9bcde	6.78de	13.12ab

Within each column, means followed by the same letter are not significantly different ($P<0.05$) using Duncan's multiple range tests.
[†] The unit of treatments is mg/L.

Effects of Bunch Thinning on Yield and Fruit Quality of 'Khalas' Date Palm

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Keywords: date palm, thinning, bunch yield, physical, chemical, characters

Abstract

'Khalas' date palm cultivar grown at the Agriculture Research and Experiment Station, Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh was thinned by removing 15 and 30% of the total number of strands from the center of each bunch. In general, the average yield per palm and per bunch for the different treatments was lower than the control treatment (non-thinning). Thinning treatments improved both physical and chemical characteristics of fruits (at beser and tamur stages), where they significantly increased fruit weight, flesh weight, fruit size, fruit length, fruit diameter, first grade of fruit percent, total soluble solids, reducing sugars, non-reducing sugar, total sugar and fruit moisture content than those of the control treatment. Removing 30% of the total number of strands from the center of each bunch, four weeks after pollination produced the highest fruit quality and could be considered as a recommended treatment in such experiment.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the important fruit species grown in the Kingdom of Saudi Arabia. 'Khalas' is one of the best soft type date palm cultivars. It can grow well under drastic environmental conditions which may be not suitable for other fruit species.

Successful orchard management practices include appropriate fruit thinning giving the remaining fruits a better chance to reach larger size and better quality.

Alternate bearing with high and low yields is common in date palms. Fruit thinning is one of the major practices that often help to overcome this problem. In addition, it gives better quality and reduces compactness among fruits within the bunch. It also helps to give a good flowering in the following year (Hussein, 1970; Moustafa et al., 1984; Khalifa et al., 1987; Marzouk et al., 2007). Such results could be attained either by reducing the number or fruits per bunch or by reducing the number of bunches per palm.

Hasan et al. (1998) found that there was an inverse relation between the number of bunches and each of volume and weight of both fruit and seed. However, the total yield of the palm showed a non linear relationship with the number of bunches. Thinning treatments improved most physical and chemical properties of fruits (Moustafa, 1998; El-Shazly, 1999). Al-Obeed et al. (2005) found that the 15% shortening of strands at pollination time led to obtain a reasonable yield with fruit quality. Removing 15% of total number of 'Haiany' and 'Halawy' bunch strands by either thinning out or cutting back before pollination was beneficial to regulate the yield with enhancing the maturity and quality of dates (Amen et al., 2007). Behseresht et al. (2007) reported that the thinning in chimiri stage had no significant effects on fruit quality and quantity when compared with that at pollination stage. Although removal of one third (control and strand-tip) of strands reduced yield, this treatment increased fruits in top grads.

MATERIALS AND METHODS

The present investigation was carried out in two successive years, 2007 and 2008,

at the Research and Agriculture Experimental Station at Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh. Five 10-year-old date palms grown on sandy soil were selected. The experimental palms were healthy, uniform in growth, vigor and height. Pollination was achieved by using pollen grains from the same parent in both seasons. All cultural practices were carried out according to the normal schedule for experimental palms. Only 10 bunches were left on each experimental tree. Thinning treatments were applied 30 days after hand pollination in both seasons as follows: control (unthinning), removing 15 and 30% of the total number of strands from the center of each bunch.

Experimental treatments were arranged in randomized complete block statistical design with five replications (one palm tree for each replication).

The yield of experimental trees was harvested through the first half of August. Average bunch weight in kg/palm was recorded. Samples of 10 date fruits were picked at random for the determination of both fruit physical and chemical characteristics.

Average Bunch Yield

Average bunch yield was estimated by kg.

Fruit Physical Characters

Samples of five replicates, each 10 fruits were taken randomly from each bunch to determine fruit size and dimensions (length and diameter, in cm), fruit weight, fruit flesh weight and seed weight (in g).

Chemical Properties

Chemical properties of fruits (moisture content, total soluble solid TSS), fruit acidity and sugar content (reducing, non-reducing, and total sugar) were determined according to AOAC (1995).

Statistical Analysis

All collected data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1980). Means were compared by the Least Significant Difference test (LSD) at the 5% level of probability in the two seasons of experiment.

RESULTS AND DISCUSSION

Bunch Yield (kg)

Data presented in (Table 1) show the average bunch weight of 'Khalas' date palm. Fruit thinning of 'Khalas' palm significantly decreased bunch yield compared to the control treatment in both seasons. Meanwhile, the reduction in bunch yield was increased by increasing the thinning percent. Nevertheless, differences between various degrees of thinning were too small to reach the significant level. These results are in agreement with those published by Hussein (1970), Miremadi (1970), Mustafa et al. (1984), Mustafa (1993), El-Shazly (1999), Osman, and Soliman (2001) on several date cultivars, since removal of part of the strands led to less bunch weight.

Fruit Characteristics

1. Physical Properties. Data concerning the physical properties of fruits in the two seasons are presented in Tables 1 and 2.

Fruit Weight (g). Data indicated that all treatments of thinning significantly increased the average fruit weight of 'Khalas' date palm as compared to that of the control (at beser and tamur stages) in both seasons. The increase in average fruit weight which was achieved by thinning may be due to the reduction in fruits compactness which prevents their accumulation within bunch. Consequently, such fruits take the opportunity of natural growth. Comparing the effect of thinning treatments on fruit weight, it was found that removing 30% of the total strands from the bunch center significantly increased the

average fruit weight compared to other thinning treatments in both seasons. The obtained results are similar to those of Hassaballa et al. (1983), Mustafa et al. (1984), Khalifa et al. (1987), Mustafa (1993), Osman and Soliman (2001) and Al-Obeed et al. (2005). They all reported that fruit thinning increased the fruit weight of date palm.

Seed Weight (g). Results indicate that the seed weight was not significantly affected by thinning treatments for 'Khalas' (at beser and tamur stages) in both seasons. These findings are in partial agreement with those achieved by El-Shazly (1999).

Flesh Weight (g). The flesh weight differs significantly among treatments. All thinning treatments significantly increased fruit flesh weight (at beser and tamur stages) compared to the control in both seasons. Meanwhile, trees which were thinned by removing 30% of the total strands from the bunch center showed the highest pulp weight of fruits as compared to removing 15% of the total strands from the bunch center. The results are in line with those of Hassaballa et al. (1983), Mustafa et al. (1984), Khalifa et al. (1987), Mustafa (1993), Osman and Soliman (2001) and Al-Obeed et al. (2005).

Fruit Size (cm^3). Concerning the fruit size, the data indicate a significant difference in both seasons. Removing 30% of the total strands from the bunch center gave the highest fruit size (at beser and tamur stages) as compared to the control and other studied treatments in both seasons. These results are in agreement with those reached by Mustafa (1993), Osman and Soliman (2001) and Al-Obeed et al. (2005).

Fruit Dimensions. Data of fruit dimensions (length and diameter) for 'Khalas' during the beser stage in both seasons showed that fruit length and diameter were increased significantly by increasing the thinning degree. Data indicated that trees thinned by 30% produced the significantly maximum increase in average fruit length and diameter in both seasons. Similar effects of fruit thinning on fruit dimensions (length and diameter) were reported by other investigators on several date cultivars by Hussein (1970), Hussein et al. (1976), Khalifa et al. (1987), Mustafa (1993), Osman and Soliman (2001) and Al-Obeed et al. (2005).

First and Second Fruit Grade. Concerning the fruit grading, thinning treatments produced a higher percent of first grade fruit than the unthinned treatment (control). Moreover, removing 30% of strands/bunch resulted in the highest percent of first grade fruits compared to 15% and control treatment (56.98 and 61.57, 49.44 and 49.35, 42.87 and 42.63%) in the first and second seasons, respectively. Meanwhile, more second grade fruits were shown in the control followed by 15 and 30% of thinning treatments (57.12 and 57.37, 50.56 and 50.64, 43.02 and 38.43%) in both seasons, respectively.

2. Chemical Properties. Data concerning the chemical properties of fruits in the two seasons are presented in Table 3.

Total Acidity (%). Regarding the investigated treatments, the fruit acidity percentage was not influenced significantly by thinning treatments in the two seasons. The present data are in agreement with El-Shazly (1999) on 'Nabtet Ali' and Osman and Soliman (2001) on 'Sakkoti', 'Shamia' and 'Balady' dates.

Total Soluble Solids (%). Data clearly indicated that different thinning treatments had increased TSS% in fruit compared to the control in both seasons. The most effective treatment in such concern was thinning of 30% of the total number of strands. In general, these results agree with those found by El-Shazly (1999), Osman and Soliman (2001) and Al-Obeed et al. (2005).

3. Sugar Contents.

Reducing Sugars (%). Concerning the effect of thinning treatments on reducing sugars, data indicated that removing 30% of the total strands from the bunch center gave the highest reducing sugars (at tamur stage) as compared to the control and the other studied treatment in both seasons. These results are in general agreement with those obtained by El-Kassas (1983), Khalifa et al. (1987), Sayed (1991) on 'Zaghloul' dates, El-Shazly (1999) on 'Nabtet Ali' dates, Osman and Soliman (2001) on 'Sakkoti', 'Shamia' and 'Balady' dates and Al-Obeed et al. (2005) on 'Succary' dates. They reported that fruit thinning treatments increased reducing sugars.

Non-Reducing Sugars (%). The obtained data indicated that, the non-reducing sugars

percentage shows a similar trend as reducing sugars. The non-reducing sugars were increased significantly by removing 30% of the total strands from the bunch center (tamur stage) than the control and the other studied treatment. These results are in agreement with those published by Osman and Soliman (2001) and Al-Obeed et al. (2005).

Total Sugars (%). Values of total sugars % followed a trend similar to that of total soluble solids percent in both seasons. Thinning 30% of the total number of strands significantly increased total sugars percent (tamur stage) than the control and thinning 15% treatment. In general, these findings concerning the response of 'Khalas' fruit chemical characteristics to the different treatments of fruit thinning goes in line with those found by El-Kassas (1983), Khalifa et al. (1987) and Sayed (1991). They mentioned that fruit thinning increased total sugars of 'Zaghloul' dates. In addition, Hussein et al. (1992) reported that fruit sugar contents of 'Samani' dates significantly increased by fruit thinning. Similar results were found by Mustafa (1993) on Siwi and El-Shazly (1999) on 'Nabtet Ali' dates. They found that removing 30% of entire spikelets from bunches center of fruit dates increased the fruit total sugars content. Al-Obeed et al. (2005) on 'Succary' dates found that the shortening of strands by 40% gave the highest value of total sugars.

4. Moisture Content (%). Regarding the moisture, the results indicated significant differences in both seasons. Since removing 30% of the total strands from the bunch center gave the highest moisture content (tamur stage) as compared to the control and other studied treatment in both seasons. On the contrary, Osman and Soliman (2001) and Al-Obeed et al. (2005) reported that the moisture content was not significantly affected by thinning treatments or the control which recorded highest values as compared to other thinning treatments.

In conclusion, thinning treatments improved fruit characteristics where they significantly increased fruit physical and chemical properties of dates (at tamur stage) compared to the control. Meanwhile, the most beneficial treatment in this regard was thinning 30% of the total number of strands from the center of bunches.

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Tables

Table 1. Bunch weight and physical characteristics (tamur stage) of 'Khalas' date palm in the 2007 and 2008 seasons.

Treatments	Bunch yield (kg)		Fruit weight (g)		Seed weight (g)		Flesh weight (g)	
	2007	2008	2007	2008	2007	2008	2007	2008
Control	12.00	13.25	8.64	7.28	0.78	0.87	7.86	6.41
Thinning 15%	10.21	11.34	9.75	8.02	0.81	0.98	8.94	7.04
Thinning 30%	9.37	10.75	10.07	8.70	0.90	0.83	9.17	7.87
LSD	1.12	0.54	0.69	0.65	NS	NS	0.69	0.91

Table 2. Fruit physical characteristics (beser stage) of 'Khalas' date palm.

Treatments	Fruit weight (g)	Seed weight (g)	Flesh weight (g)	Fruit volume (cm)	Fruit length (cm)	Fruit diameter (cm)	Grade 1 (%)	Grade 2 (%)
				2007				
Control	10.01	1.18	8.83	10.00	2.53	2.31	42.87	57.12
Thinning 15%	11.43	1.19	10.24	11.50	2.62	2.39	49.44	50.56
Thinning 30%	12.31	1.19	11.12	12.35	3.84	2.59	56.98	43.02
LSD	0.64	NS	0.71	1.40	0.16	0.16	2.10	3.33
				2008				
Control	10.20	1.17	9.03	9.83	3.53	2.24	42.63	57.37
Thinning 15%	12.38	1.19	11.19	12.20	3.72	2.37	49.35	50.64
Thinning 30%	12.66	1.27	11.39	12.30	3.75	2.39	61.57	38.43
LSD	0.52	NS	0.42	0.74	0.17	0.11	3.32	3.32

Table 3. Fruit chemical characters (tamur stage) of 'Khalas' date palm.

Treatments	TSS (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Acidity (%)	Moisture content (%)
			2007			
Control	54.82	30.22	15.13	45.35	0.176	15.64
Thinning 15%	58.40	31.27	16.58	47.85	0.167	16.29
Thinning 30%	60.40	33.65	17.44	51.09	0.157	17.89
LSD	3.01	0.76	3.81	3.38	NS	1.10
			2008			
Control	56.00	32.38	16.78	49.16	0.255	19.96
Thinning 15%	60.60	34.08	18.70	52.78	0.230	20.06
Thinning 30%	63.10	37.48	19.23	56.71	0.204	21.49
LSD	2.43	1.31	1.34	0.66	NS	1.41

Some Physical Characteristics of Several Libyan Date Palm Cultivars

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Keywords: *Phoenix dactylifera* L., Libya, spikes, fruits

Abstract

Eight date palm cultivars (*Phoenix dactylifera* L.) grown in Sabha, Libya, namely: 'Talees', 'Tasfert', 'Taghiat', 'Adhwi', 'Amreer', 'Bustian', 'Sotrah' and 'Asber' were included in this study. Five 8-10-year-old trees were randomly chosen from each cultivar. The characteristics studied were: spike length, number of spikes/tree and number of spikelet/spike. Also 20 fruits were randomly collected from each spike to study: fruit weight, length, width and flesh thickness. Seed weight, length and width were measured too. Results showed that there were significant differences between the cultivars in the studied parameters. 'Talees' spikes were the longest (129.4 cm), 'Sotrah' and 'Amreer' were the shortest. 'Tasfert' produced the highest number of spikes/tree and 'Bustian' the lowest number. On the other hand, 'Adhwi' had the heaviest fruit (14.5 g), while 'Sotrah' fruits were the lightest (7.6 g). The results are discussed in detail.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is a palm in the genus *Phoenix*, extensively cultivated for its edible fruit (Buabady, 1998). Due to its long history of cultivation, its exact native distribution is unknown, but probably it originated somewhere in the desert oases of northern Africa, and perhaps in southwest Asia around the Arabian Gulf. However, there was archaeological evidence of date cultivation in eastern Arabia since 4000 BC (Brown, 1984).

Arabs had spread dates around South and Southeast Asia, Northern Africa, and Spain. Then dates were introduced into Mexico and California by the Spaniards in 1765 (Berbandy et al., 1998). In Libya, date palms are cultivated in three main areas: northern (coastal), central and southern regions. Each region has a different climate, and thus different cultivated date palm cultivars. Statistics for the year 1999 indicated that the number of date palms grown in Libya was six million trees, of which four million trees were under production (Ministry of Agriculture, 1999).

Because date palm can be easily grown from seed, many cultivars were developed resulting in variable vegetative characteristics. Although many of these characteristics change with the environment and agricultural practices, however, there are certain vegetative characteristics which are fixed and remain specific for each cultivar. Azziz et al. (1998) in their study of some morphological characteristics of a number of Moroccan date palm cultivars had shown that lengths of spikes, number of spikelet/spike could be used to differentiate between cultivars. Marzoky et al. (1998) studied several date palm cultivars grown in the Oman Sultanate and reported that there were significant differences between the studied varieties in number of spikes/tree. Berbandy et al. (1998) indicated that different cultivars could be differentiated by fruit characteristics which included; color, length, width and size. This was confirmed by many investigators in Egypt, Iraq, Saudi Arabia, Pakistan and Tunisia (Abdalla et al., 1993; Mohammed et al., 1983; El-Saeid et al., 1986; Khan and Khan, 1993; Buabady, 1998).

The aim of this study was to study some characteristics of eight of the most

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important date palm cultivars grown in Sabha, in the southern region of Libya namely: 'Talees', 'Tasfert', 'Taghiat', 'Adhwi', 'Amreer', 'Bustian', 'Sotrah' and 'Asber'.

MATERIALS AND METHODS

This study was conducted in The Green Band project in Sabha, Libya. Eight cultivars of date palm were used namely: 'Talees', 'Tasfert', 'Taghiat', 'Adhwi', 'Amreer', 'Bustian', 'Sotrah' and 'Asber'. Five 10-year-old trees were randomly chosen from each cultivar. They were closely alike in size, and were divided into five replicates, and each one included a tree from each cultivar. Length of spikes were measured, number of spikes/tree and number of spikelets/spike were counted. By using twenty fruits from each spike, averages of fruit weight, length, width and flesh thickness as well as averages of seed weight, length and width were determined. These characteristics were studied during ratub stages as follows:

a Fruit length was measured in cm as an average of 20 fruits randomly chosen from each spike lined up longitudinally.
b Fruit width was measured in cm as an average of 20 fruits randomly chosen from each spike lined up side by side.
c Fruit weight was determined in g as an average of 20 fruits randomly chosen from each spike.
d Fruit flesh thickness was determined at the widest part of the fruit as an average of 20 fruits randomly chosen from each spike.
e Seed characteristics which included weight, length and width were similarly studied as the fruits were.

Data were subjected to statistical analysis (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Spike Characteristics

There were significant differences in spikes length, number of spikes/tree, and spikelets/spike between the cultivars (Table 1). 'Talees' spikes were the longest (129.4 cm), 'Sotrah' and 'Amreer' were the shortest. 'Tasfert' and 'Asber' produced the highest number of spikes/tree (7.8 and 6.8 respectively) while 'Bustian' produced the lowest number (3.8) and the other cultivars were in between. 'Asbeer' spikes had a significantly higher average number of spikelets (81.8) as compared to the other cultivars.

Fruit Characteristics

There were significant differences in fruit weight, fruit length, width, and fruit flesh thickness between the cultivars (Table 2). 'Adhwi', 'Tasfert' and 'Asber' fruits were the heaviest (14.5, 12.5 and 11.2 g respectively), while 'Sotrah' fruits were the lightest (7.6 g). 'Taghiat' and 'Tasfert' produced the longest fruits (46.4 and 43.8 mm respectively), 'Sotrah' and 'Amreer' produced the shortest fruits (32.6 and 34.4 mm respectively). Fruit width was the biggest in 'Asber', 'Tasfert', 'Taghiat' and 'Amreer' (25.6, 25.4, 23.8 and 23.6 mm respectively), and lowest in 'Bustian' and 'Adhwi' (15.8 and 18.8 mm). As for the fruit flesh thickness which is the edible portion of the fruit, and thus the most important economically, was found to be in the following decending order; 'Taghiat' (8.6 mm), 'Tasfert' (7.3 mm), 'Asbeer' (7.2 mm), 'Talees' (6.2mm), 'Amreer' (5.7 mm), 'Sotrah' (5.4 mm), 'Adhwi' (4.0 mm) and 'Bestian' (3.0 mm).

Seed Characteristics

Results showed that there were significant differences between cultivars in seed weight, length, and width (Table 3). 'Adhwi', 'Tasfert' and 'Amreer' seed weight ranged between 5.4 to 6.1 g, while that of 'Sotrah' was 3.5 g only. 'Taghiat', 'Talees' and 'Tasfert' seeds were significantly the longest (32.6, 30.4 and 30.2 mm respectively) and 'Adhwi' seeds were the shortest (19.0 mm). As for seed width, 'Taghiat', 'Asbeer', 'Talees', 'Tasfert', 'Adhwi' and 'Sotrah' seeds were significantly the widest ranging

between 10.4 to 12.2 mm, while ‘Bestian’ and ‘Amreer’ seed width did not exceed 8.6 mm.

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Tables

Table 1. The averages of spike length, number of spikes/tree and number of spikelets/spike for eight date palm cultivars.

Cultivar	Spikes length (cm)	No. spikes/tree	No. spikelets/spike
Talees	129.4a	6.2bc	45.4b
Tasfert	110.2b	7.8a	32.4c
Taghait	89.6b	4.8c	49.6b
Adhwi	56.8d	5.2c	41.4b
Amreer	49.8e	4.8c	29.2c
Bustian	79.4c	3.8c	74.2b
Sotrah	46.2e	5.4b	38.4c
Asbeer	62.4c	6.8a	81.8a

Averages sharing the same letter in a column are not significantly different at 5% LSD level.

Table 2. The averages of weight, length, width and flesh width of fruits of eight date palm cultivars.

Cultivar	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit flesh width (mm)
Talees	10.5b	39.4b	20.4b	6.2a
Tasfert	12.5a	43.8a	25.4a	7.3a
Taghait	9.3b	46.4a	23.8a	8.6a
Adhwi	14.5a	25.0d	18.8c	4.0c
Amreer	9.1b	34.4c	23.6a	5.7b
Bustian	8.0b	38.8b	15.8c	3.0c
Sotrah	7.6c	32.6c	20.2b	5.4b
Asbeer	11.2a	39.2b	25.6a	7.2a

Averages sharing the same letter in a column are not significantly different at 5% LSD level.

Table 3. The averages of seed weight, seed length and seed width of eight date palm cultivars.

Cultivar	Seed weight (g)	Seed length (mm)	Seed width (mm)
Talees	4.3b	30.4a	11.4a
Tasfert	5.6a	30.2a	11.4a
Taghait	4.3b	32.6a	12.1a
Adhwi	6.1a	19.0c	10.5a
Amreer	4.6b	22.0c	8.4c
Bustian	4.1b	24.8b	8.6c
Sotrah	3.5c	25.2b	10.4a
Asbeer	5.4a	27.1b	11.8a

Averages sharing the same letter in a column are not significantly different at 5% LSD level.

Effect of Different Organic Fertilizer Sources on Improving Fruit Nutritional Value and Yield of 'Zaghloul' Dates

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Keywords: organic, inorganic, quality, nutritional value, date palm

Abstract

A field study was carried out during the 2007 and 2008 seasons on 'Zaghloul' cultivar growing in clay soil. One level of N alone or plus PK from mineral (ammonium nitrate alone or plus calcium superphosphate + potassium sulphate, NPK) and organic sources (chicken manure, cow dung and town refuse compost) were applied either alone or in combinations in order to study their influence on yield and fruit physical and chemical quality. The results revealed that organic manure either alone or combined with mineral fertilizers increased palm yield and enhanced fruit color as compared with mineral fertilization alone. Chicken manure and cow dung resulted in the best fruit weight and fruit length. Fruit TSS, anthocyanin and sugars increased and tannins content was decreased by chicken manure and cow dung as compared with combining organic manure with NPK or mineral alone. In general, micronutrients contents were significantly higher in fruits by applying organic manure alone than organic manure combined with NPK or mineral fertilization alone. Organic manure fertilization alone (especially CM and CD) resulted in decreasing fruit Pb and Cd contents compared to mineral fertilization.

INTRODUCTION

Date palm *Phoenix dactylifera* L., is one of the oldest fruit trees in the world, known as the tree of life because of its resilience, its need for limited water inputs, its long term productivity and its multiple purpose qualities. According to FAO (2009), Egypt is considered the first country of the top ten date producers (1,130,000 tons). 'Zaghloul' date is the most economically important soft cultivars grown in Egypt. Most of the date palms in Egypt are growing in loam and sandy loam soils. With time these types of soils may become deficient in N, P, K, Mg and B (Tisdale and Nelson, 1978). Fertilization programs play an effective role in increasing palm yield and improving date quality. The use of chemical fertilizer is necessary for supplying the nutrient requirements. However, the continual use of chemical fertilization leads to deterioration of soil characteristics and fertility (Shimbo et al., 2001). Also, it is reported that chemical fertilizers such as super phosphate contain Cd and Pb and they may be the major source of Cd uptake in plants (Shimbo et al., 2001). Thus, continuous use of chemical fertilizer might lead to the accumulation of heavy metals in plant tissues which contributes to fruit nutrition value and edible quality. The second source of nutrients is organic manure which is derived from animal or plant sources. It is an excellent source of organic matter and macro- and micro-nutrients. Animal manure is an important source of N, P and K and its addition to the soil increases the available P and exchangeable K, Ca and Mg contents (Magdoff, 1998). Application of organic fertilizers improved structural stability and lowered bulk density of the soil, improved moisture retention, water infiltration rate and the hydraulic conductivity of soil (Tisdale et al., 1990; Young, 1997). Also, manures were found to enhance soil biological properties (Chai et al., 1988) and soil fertility leading to an increase in crop yield (Lal and Mathur, 1989). However, organic manure may be

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beneficial to crop and soil on the long term (Tirol-Padre et al., 2007), and their efficiency in enhancing crop growth and yield in the short term by combining them with mineral fertilizers has been reported (Kanal and Kuldkepp, 1993; Mottaghian et al., 2008; Aisueni et al., 2009).

In accordance to the mentioned above the present study was undertaken to investigate the effect of different nutrient supply regimes namely, cow dung, poultry manure, compost and mineral fertilizer on the yield and fruit quality and nutritional value of 'Zaghloul' date palm.

MATERIALS AND METHODS

Plant Material and Experimental Design

A field experiment was conducted during the 2007 and 2008 seasons in a private orchard at El-Tarh region, El-Behera Governorate, Egypt on 26-year-old 'Zaghloul' date palm planted at 10×10 m apart. The soil was clay, well drained with water table 110 cm and pH 8. The palms were fertilized with inorganic and/or organic fertilizers either alone or in combinations. The inorganic fertilizer was ammonium nitrate (33.5% N) + (triple super phosphate, 46% P_2O_5) + potassium sulphate (48% K_2O). Organic fertilizer was poultry manure (chicken manure, CM), cattle manure (cow dung, CD), and compost (town refuse, TR). Chemical analysis (average of both seasons) of organic fertilizers used is presented in Table 1. Each treatment included an amount of 1000 g nitrogen (applied from inorganic or organic source alone, or in combinations) + 535 g K_2O + 500 g P_2O_5 (estimated from the average amount of K_2O and P_2O_5 determined in the three organic manures). In both seasons mineral fertilizer was added at three intervals; half amount in March, a quarter in May and the last quarter in August of both seasons, and organic fertilizers were applied once at the second week of December. Female palms were selected similar in growth, vigor, height, pollen source and age (30 years old) and were subjected to the normal cultural practices carried out as usual used for date palms. Eight soil application treatments were arranged in a complete randomized design with five replicates (1 replicate = palm) per treatment (i.e., 5×8=40 palms). The treatments were as follows: T1) 3 kg ammonium nitrate + 1 kg triple super phosphate + 1 kg potassium sulphate (NPK mineral only); T2) 3 kg ammonium nitrate (N only); T3) 30 kg chicken manure (CM); T4) 45 kg cow dung (CD); T5) 75 kg town refuse (TR); T6) ½ NPK mineral + ½ CM (½ T1 + ½ T3); T7) ½ NPK mineral + ½ CD (½ T1 + ½ T4); T8) ½ NPK mineral + ½ TR (½ T1 + ½ T 5).

Yield and Fruit Physical Characteristics

The yield was harvested at mid-October of both seasons and the average yield and bunch was recorded in kg. A 25 fruit sample from each replicate was taken to determine average fruit weight (g), length (cm) and diameter (cm). Also fruit color was recorded for each fruit sample using a degree of color intensity as follows: (1)=100% green, (2)=25% red, (3)=50% red, (4)=75% red and (5)=100% red.

Fruit Chemical Characteristics

In a 20 fruit sample the percentage of juice TSS was determined using a hand refractometer. Soluble tannins content (% on fresh weight basis) and total and reducing (% on fresh weight) were determined according to AOAC (1995). Anthocyanin content in the fruit peel was measured using a spectrophotometer by the method of Fuleki and Francis (1968).

Fruit Mineral Content

A sample of 25 fruits for each replicate was washed with tap water, rinsed twice in distilled water, dried to a constant weight in an air drying oven at 70°C, ground and digested with H_2O_2 and H_2SO_4 according to Evanhuis and De Waard (1980). Total nitrogen was determined calorimetrically according to Evanhuis (1976). P (%) was

determined colorimetrically by ascorbic acid method according to Murphy and Riley (1962). K (%) was determined by flame photometer. Pb, Cd, Ca, Mg, Fe, Zn and Mn contents (ppm), Ca and Mg (%) were measured using an atomic absorption spectrophotometer 305B.

Statistical Analysis

All data were tested for treatments effects on analyzed parameters by analysis of variance ANOVA using the Statistical Analysis System (SAS, 1990).

RESULTS AND DISCUSSION

Yield

The data of both seasons presented in Table 2 showed that palm yield was significantly higher by applying CM, CD, ½ NPK mineral + ½ CM, ½ NPK mineral + ½ CD and ½NPK mineral + ½ TR than NPK or N mineral alone and TR. Chicken manure resulted in the highest yield as compared with the other treatments in the first season only. Our results support earlier findings which indicated the importance of supplementing the organic matter with mineral fertilizers to increase yield of date palms (Al-Bakr, 1982; Bacha and Abo-Hassan, 1983).

Fuit Physical and Chemical Characteristics

The data in Table 2 showed that a significant enhancement in fruit color was obtained in both seasons by applying CM, CD, TR and ½ NPK mineral + ½ CM when compared with NPK mineral or N alone, with no significant differences obtained among them. In addition, ½ NPK mineral + ½ CD and ½ NPK mineral + ½ TR increased fruit color compared to N mineral alone. Average fruit weight of both seasons did not significantly differ among all treatments (except N mineral in both seasons). Applying CM, CD, TR and ½ NPK mineral + ½ CD gave significantly higher fruit weight than N mineral alone in both seasons. Also, NPK mineral (in the second season), ½ NPK mineral + ½ CM and ½ NPK mineral + ½ TR (in the first season) resulted in higher fruit weight than N mineral alone. In both seasons fruit length was significantly higher than N mineral alone by all treatments (except town refuse in the first season). In addition, application of CM resulted in higher fruit length than N mineral alone (in both seasons) and NPK mineral, TR, ½ NPK mineral + ½ TR in the first season). Also, application of CD alone resulted in higher fruit length than ½ NPK mineral + ½ TR in both seasons. Fruit diameter did not significantly differ between all treatments (except N mineral and TR) and NPK mineral in both seasons. However, NPK mineral obtained a higher fruit diameter than N mineral and TR treatments in both seasons.

In addition, the data in Table 3 show that application of CM, CD, TR, ½ NPK mineral + ½ CM, ½ NPK mineral + ½ CD and ½ NPK mineral + ½ TR resulted in higher TSS than NPK or N mineral treatments (except ½ NPK mineral + ½ CM and ½ NPK mineral + ½ CD in the first season). In addition, the application of organic fertilizers either alone or in combinations with the NPK mineral fertilizer resulted in higher fruit total sugars than mineral fertilization alone (NPK or N mineral alone) in both seasons. However, only the application of CM or CD gave higher non-reducing sugars content than NPK mineral in the first season only. Fruit peel anthocyanin content increased significantly by the application of organic fertilizers alone (CM, CD or TR) when compared with the mineral fertilizer treatments (NPK or N alone) in the first season only. The CD fertilizer resulted in higher anthocyanin content than all organic manure fertilizers combined with NPK mineral in both seasons. In addition, the data obtained of both seasons showed no significant difference in fruit pulp tannins content among all fertilizer applications except for N mineral. The application of N mineral alone resulted in significantly higher content than TR, ½ NPK mineral + ½ CD and ½ NPK mineral + ½ TR (in the first season), and than all treatments (in the second season).

The above results indicated enhancement in fruit quality characteristics by the

application of organic manures either alone or when supplemented with mineral fertilizers. These results are in line with those reported by Bacha and Abo-Hassan (1983). In addition, Al-Kharusil et al. (2009) obtained the highest dry fruit matter content by combining NPK mineral with organic peat. In our study the application of N by combination of organic sources gave better fruit characteristics than using mineral source alone. Similarly, Sharawy (2005) reported that the combined application of N through mineral and compost was effective in improving the fruit quality of lime trees.

Fruit Mineral Content

The data presented in Table 4 showed that in the first season only CM and ½ NPK mineral + ½ TR increased fruit N content compared to NPK mineral, whereas, in the second season fruit N was significantly lower by adding N mineral, CD and ½ NPK mineral + ½ TR than NPK mineral. Moreover, no significant difference was obtained between NPK mineral and, TR, ½ NPK mineral + ½ CM and ½ NPK mineral + ½ CD in both seasons. P content was higher by N alone fertilization than NPK in both seasons. Moreover, in the first season only TR, ½ NPK mineral + ½ CM resulted in significantly higher P than NPK mineral, whereas, in the second season all fertilization increased P content in comparison with NPK mineral alone. K content increased by the N mineral alone and TR in both seasons, ½ NPK mineral + ½ CM and ½ NPK mineral + ½ TR (in the first season) CD and ½ NPK mineral + ½ CD (in the second season) as compared with applying NPK mineral. In addition, the data of both seasons showed no significant difference when CM and CD fertilizers were applied either alone or combined with NPK mineral. Moreover, only mineral N alone in the first season and ½ NPK mineral + ½ CM in the second season gave higher fruit Ca content than NPK mineral. In the first season, only CD increased Mg content as compared with NPK mineral, whereas, in the second season all treatments (except N alone) increased Mg content as compared with NPK mineral. In addition, CD gave significantly higher Fe content than NPK mineral in both seasons. Also, CM (in the first season) and ½ NPK mineral + ½ CM (in the second season) had significant higher Fe content than NPK mineral. Fruit Zn content was significantly higher by application of CM, TR, ½ NPK mineral + ½ CD and ½ NPK mineral + ½ TR than NPK or N mineral in both seasons. Mn content increased by CD and ½ NPK mineral + ½ CM as compared with NPK mineral in the first season, whereas, in the second season all treatments (except ½ NPK mineral + ½ CD) increase Mn as compared with NPK mineral and N alone. Fruit Cu was increased by all treatments (except N alone) as compared with NPK mineral in the first season only. The application of organic fertilizers either alone or in combination with NPK mineral did not significantly differ from each other in fruit Ca, Mg, Fe, Zn and Mn contents in both seasons.

The general increase in fruit mineral contents as a result of applying organic manures in combinations with mineral fertilizers might be due to the enhancement of soil properties and soil fertility by organic soil amendments (Mathew and Karikari, 1995; Kaurch et al., 2005) which might lead to the increase of available nutrients and their uptake (Kanal and Kuldkepp, 1993).

Data presented in Table 4 also indicated that fruit Pb content was significantly decreased in fruits fertilized with the three organic manures (CM, CD and TR) alone as compared with NPK mineral (in the first season) and N alone (in both seasons). In addition, the application of organic fertilizers alone resulted in lower fruit Pb content than combining them with the mineral NPK fertilizer in both seasons. Moreover, data of both seasons showed that fruit Cd content did not differ significantly when organic fertilizers were applied either alone or in combinations (except ½ NPK mineral + ½ CM and ½ NPK mineral + ½ TR in the second season) as compared with NPK mineral. No significant differences were obtained between organic fertilization alone or in combined with mineral fertilizer (except that combining TR manure with mineral NPK resulted in higher Cd than TR alone in the second season only). In general using mineral nitrogen fertilizer alone gave the highest Pb and Cd contents in comparison with all other fertilizing treatments in

both seasons. In our study, the application of organic fertilizers alone resulted in lower fruit Cd and Pb contents than applying them in combinations with mineral fertilizer or mineral fertilizer alone. Shimbo et al. (2001) stated that chemical fertilizers such as super phosphate contained Cd and Pb and they can be the major source of their uptake in plant.

CONCLUSION

From the mentioned above we concluded that applying organic fertilization to 'Zaghloul' date palms improves fruit quality and also resulted in better nutritional quality of the fruit than the mineral fertilization as it lowered the amount of heavy (Pb and Cd) contents as compared with mineral fertilizing only.

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Tables

Table 1. Chemical analysis of organic fertilizers used (average of both seasons 2007 and 2008).

Organic fertilizers	Parameters (%)				
	Moisture	Organic matter	Total N	P_2O_5	K_2O
Chicken manure (CM)	9.32	33.54	3.38	1.68	1.72
Cow dung (CD)	22.98	47.69	2.25	1.11	1.21
Town refuse (TR)	33.36	58.43	1.35	0.64	0.72

Table 2. The effect of organic and inorganic fertilization on 'Zaghloul' yield and fruit physical characteristics in the 2007 and 2008 seasons.

Treat.	Yield (kg/palm)		Fruit color		Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
T1	182_{c}	170_{b}	4.6_{bc}	4.7_{c}	33.0_{ab}	35.7_{a}	6.3_{bcd}	6.9_{ab}	2.9_{a}	2.8_{ab}
T2	178_{c}	159_{b}	4.5_{c}	4.5_{d}	30.3_{b}	31.7_{b}	5.8_{e}	5.4_{c}	2.1_{c}	2.0_{cd}
T3	208_{a}	189_{a}	5.0_{a}	5.0_{a}	34.9_{a}	35.6_{a}	6.8_{a}	7.2_{a}	2.7_{abc}	3.0_{a}
T4	198_{b}	184_{a}	4.9_{a}	5.0_{a}	34.8_{a}	36.3_{a}	6.6_{ab}	7.4_{a}	2.9_{ab}	3.0_{a}
T5	179_{c}	166_{b}	4.9_{a}	4.9_{ab}	32.6_{ab}	34.9_{a}	5.9_{de}	6.7_{ab}	2.2_{bc}	1.8_{d}
T6	193_{b}	184_{a}	5.0_{a}	4.9_{ab}	32.7_{ab}	34.1_{ab}	6.4_{abc}	6.8_{ab}	2.8_{ab}	2.8_{ab}
T7	200_{b}	186_{a}	4.8_{ab}	4.8_{bc}	34.0_{a}	36.0_{a}	6.7_{ab}	6.8_{ab}	2.9_{a}	2.9_{ab}
T8	197_{b}	185_{a}	4.8_{ab}	4.8_{bc}	32.4_{ab}	34.0_{ab}	6.1_{cde}	6.4_{b}	2.6_{abc}	2.4_{bc}

Values within a column with the same letter are not significantly different ($p<0.05$).

Table 3. The effect of organic and inorganic fertilization on ‘Zaghloul’ fruit chemical characters in the 2007and 2008 seasons.

Treatment	TSS (%)		Reducing sugars (%)		Total sugars (%)		Anthocyanin (mg/100 g fresh weight)		Tannins (%)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
T1	24.9_{c}	26.4_{b}	17.0_{c}	16.1_{d}	22.1_{d}	22.5_{b}	16.3_{cd}	18.5_{a}	0.15_{ab}	0.19_{b}
T2	25.6_{bc}	23.6_{b}	15.8_{c}	15.2_{d}	21.9_{d}	20.3_{b}	14.4_{d}	16.3_{bc}	0.17_{a}	0.23_{a}
T3	28.9_{a}	30.9_{a}	19.1_{b}	20.8_{abc}	26.2_{bc}	27.5_{a}	20.3_{ab}	18.4_{a}	0.13_{ab}	0.16_{b}
T4	29.1_{a}	30.9_{a}	19.6_{ab}	20.0_{bc}	28.0_{a}	27.0_{a}	22.7_{a}	19.1_{a}	0.11_{ab}	0.18_{b}
T5	28.9_{a}	31.9_{a}	21.0_{a}	21.0_{abc}	26.1_{bc}	27.8_{a}	19.8_{b}	17.9_{ab}	0.09_{b}	0.18_{b}
T6	27.7_{ab}	32.2_{a}	20.2_{ab}	22.7_{a}	25.3_{c}	27.9_{a}	17.8_{bc}	14.0_{d}	0.12_{ab}	0.16_{b}
T7	27.4_{ab}	30.8_{a}	20.0_{ab}	19.4_{c}	24.9_{c}	26.9_{a}	18.9_{bc}	15.3_{cd}	0.10_{b}	0.19_{b}
T8	29.0_{a}	31.2_{a}	19.8_{ab}	22.2_{ab}	27.1_{ab}	28.5_{a}	18.2_{bc}	14.3_{d}	0.10_{b}	0.06_{c}

Values within a column with the same letter are not significantly different ($p<0.05$).

Table 4. The effect of organic and inorganic fertilization on 'Zaghloul' fruit mineral content in the 2007and 2008 seasons.

Treatment	N	P	K	Ca	Mg	Fe	Zn	Mn	Pb	Cd
			(%)					(ppm)		
					2007					
T1	1.02_{cde}	0.09_{d}	0.69_{b}	0.61_{b}	0.39_{bc}	55_{b}	35_{b}	37_{b}	1.08_{ab}	0.010_{b}
T2	0.96_{e}	0.19_{a}	0.98_{a}	0.78_{a}	0.34_{c}	52_{b}	30_{b}	38_{ab}	1.17_{a}	0.018_{a}
T3	1.23_{a}	0.11_{cd}	0.78_{ab}	0.63_{ab}	0.47_{a}	66_{a}	41_{a}	40_{ab}	0.89_{c}	0.010_{b}
T4	1.01_{de}	0.12_{bcd}	0.80_{ab}	0.59_{b}	0.42_{ab}	68_{a}	45_{a}	43_{a}	0.94_{c}	0.009_{b}
T5	1.12_{bc}	0.15_{b}	0.97_{a}	0.59_{b}	0.45_{ab}	57_{b}	44_{a}	39_{ab}	0.78_{d}	0.008_{b}
T6	1.05^{bcde}	0.13_{bc}	0.90_{a}	0.61_{b}	0.39_{bc}	54_{b}	34_{b}	43_{a}	1.09_{ab}	0.013_{ab}
T7	1.08_{bcd}	0.10_{cd}	0.81_{ab}	0.58_{b}	0.46_{ab}	52_{b}	41_{a}	40_{ab}	1.06_{b}	0.011_{ab}
T8	1.14_{ab}	0.10_{cd}	0.90_{a}	0.62_{b}	0.40_{abc}	57_{b}	42_{a}	36_{b}	1.12_{ab}	0.010_{b}
					2008					
T1	1.19_{ab}	0.09_{e}	0.71_{c}	0.57_{b}	0.35_{b}	58_{c}	28_{d}	31_{f}	1.01_{bcd}	0.009_{c}
T2	1.03_{d}	0.13_{c}	0.91_{ab}	0.68_{ab}	0.35_{b}	50_{d}	29^{d}	33_{ef}	1.22_{a}	0.020_{a}
T3	1.13_{bc}	0.14_{c}	0.82_{bc}	0.64_{ab}	0.44_{a}	63_{abc}	33_{c}	40_{ab}	1.00_{bcd}	0.012_{bc}
T4	1.09_{cd}	0.11_{d}	0.99_{a}	0.67_{ab}	0.40_{a}	69_{a}	30_{cd}	41_{a}	0.98_{cd}	0.010_{c}
T5	1.17_{b}	0.14_{c}	0.99_{a}	0.63_{ab}	0.41_{a}	62_{bc}	39_{b}	40_{ab}	0.89_{d}	0.010_{c}
T6	1.19_{ab}	0.17_{b}	0.80_{bc}	0.77_{a}	0.40_{a}	65_{ab}	40_{b}	38_{bc}	1.08_{bc}	0.015_{b}
T7	1.26_{a}	0.19_{a}	0.90_{ab}	0.69_{ab}	0.42_{a}	64_{abc}	44_{a}	34_{de}	1.11_{ab}	0.013_{bc}
T8	1.07_{cd}	0.13_{c}	0.81_{bc}	0.68_{ab}	0.44_{a}	60_{bc}	38_{b}	36_{cd}	1.09_{bc}	0.016_{ab}

Values within a column with the same letter are not significantly different ($p<0.05$).

Effect of Some Growth Regulators on Yield and Fruit Quality of 'Samani' Date Palm

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Keyword: date palm, growth regulators, yield, fruit quality

Abstract

This study was carried out during two successive seasons (2006 and 2007) at the Experimental Research Station, Faculty Agriculture Giza, Egypt. Different GA_3 and BA (0, 50, 100 and 150 ppm) and CPPU concentrations (0, 5, 10, and 20 ppm) were sprayed on 'Samani' date palm to study their effect on yield and fruit characteristics. GA_3 and BA applications at the higher concentrations increased average bunch weigh, average fruit weigh, flesh weight, fruit length, fruit diameter, total soluble solids percentage, total sugars percentage and decreased total acidity percentage. CPPU applications gave the lowest total soluble solids, total sugars and increased total acidity. It could be concluded that GA_3 and BA at a concentration of 150 ppm gave the highest yield with the best quality and fruit characteristics.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is considered one of the leading fruit crops. 'Samani' is the most important cultivar of soft dates in Egypt. Fruit thinning often helps overcoming alternate bearing and improves fruit quality (Al-Bakr, 1972). Growth regulating substances as a factor affecting the physiology of plant growth and development have been used extensively on horticultural crops. Several investigators have studied the effect of GA_3, BA and CPPU on yield and fruit quality. The effect of gibberellic acid (GA_3) on yield and fruit characteristics of dates was reported by Hussein et al. (1976), El-Nabawy et al. (1977), Mougheith and Hasaballa (1979), Maximos et al. (1980), Abou Aziz et al. (1982), Hassaballa et al. (1984), Wazir (1985), El-Kassas (1986), Mohamed et al. (1986), Tafazoli (1991), El-Hodairi et al. (1991) and Abo El-Ez et al. (2002).

Benzyladenine is an effective thinner at concentrations of 50-200 for apple, it is effective when applied at the 10-mm stage of fruit development (Greene, 1993). Biasi et al. (1993) showed that field application of the cytokinin-like compound CPPU markedly improves fruit size of 'Granny Smith' apple, it promotes cell division and increases fruit size when applied during the first stages (15 days after full bloom) of apple fruits' development.

Therefore, this investigation was carried out to study the effect of spraying different concentrations of gibberellic acid (GA_3), 6-Benzyladenine (BA) and N-(2-chloro-4-pyridyl)-N-phenyl urea (CPPU) on the fruit retained percentage, yield and fruit quality of 'Samani' date palm grown in Giza governorate, Egypt.

MATERIALS AND METHODS

This study was carried out during two successive seasons (2006 and 2007) on 'Samani' date palm grown in a sandy clay loam soil at the Experimental Research Station, Faculty Agriculture, Giza Governorate, Egypt. Thirteen date palm trees were selected similar in growth, vigor, height, age (10 years old), moderate pruning (10:1 leaf/bunch ratio. Pollination was achieved by using pollen grains from the same male palm grown in the same region in both seasons. The number of spathes per palm was adjusted to 6

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bunches by removing excess earliest.

This experiment was carried out to study the effect of spraying some growth regulators (gibberellic acid (GA_3), 6-Benzyladenine (BA) and CPPU (Citofex)) on yield, fruit physical and chemical charcteristics. Date palm trees were sprayed two weeks after pollination, and divided into 10 treatments in three replicates (each of one tree) and arranged in a randomized complete block design as the following spray treatments: 1) control (water spray); 2) GA_3 (50 ppm); 3) GA_3 (100 ppm); 4) GA_3 (150 ppm); 5) BA (50 ppm); 6) BA (100 ppm); 7) BA (150 ppm); 8) CPPU (5 ppm); 9) CPPU (10 ppm); 10) CPPU (20 ppm).

The yield of fruits for this experiment was harvested at the peak of colour development and the following determinations were carried out:

Fruit Retained Percent

Fruit retained percent was calculated at harvest date. The average bunch weight was estimated as kg.

Yield and Fruit Physical Properties

Yield as (kg/tree), samples of 10 fruits were taken randomly from each bunch to determine fruit weight (g), fruit size (cm^3), fruit length and diameter (cm) and fruit flesh weight (g).

Fruit Chemical Properties

The percentage of TSS was determined in the fruit juice using a hand refractometer. Fruit acidity was determined according to AOAC (1985). Total and reducing sugars were determined according to Smith et al. (1956). Non-reducing sugars were determined by the difference between total and reducing sugars.

Statistical Analysis

A split-plot design in complete randomized block design with three replications was used. The dates were assigned to the main plots, while the treatments ranked to the sub-plots. The differences between means were compared using New LSD values at 0.05 level of significance, according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Fruit Retained Percentage

Data presented in Table 1 show the effect of GA_3, BA and CPPU on the percentage of retained fruits of 'Samani' date palm. Data indicated that the highest percentage of retained fruits was noticed in untreated trees (48.52 and 42.56%), while GA_3 100 ppm, BA 150 ppm and GA_3 150 ppm gave the lowest percentage of retained fruits (26.13, 28.31, 28.62 and 25.17, 26.92, 23.52%) in the first and second season respectively.

Bunch Weight

Concerning the average bunch weight, Table 1 indicates that different concentrations of GA_3, BA and CPPU significantly affected bunch weight in both seasons. GA_3 at150 ppm gave the highest bunch weight (24.81 and 23.52 kg) in the first and second season, respectively, however CPPU at 5 and 10 ppm gave the lowest bunch weight, 18.64 and 18.36 kg in the first season, but in the second season CPPU at 5 ppm only gave the lowest value (17.21 kg).

Yield

Data in Table 1 show yield as kg/tree of 'Samani' date palm. It could be noticed that trees treated by GA_3 at 150 ppm gave the highest significant yield (kg/tree) during the 1^{st} (148.9 kg/tree) and 2^{nd} season (130.8 kg/tree), respectively. While trees treated by

CPPU at 5 ppm significantly showed the least yield values (18.64 and 103.26 kg/tree) in both seasons respectively.

The previous results are in harmony with those obtained by many investigators such as El-Salhy (1975), Mougheith and Hassaballa (1979), Abou-Aziz et al. (1982), Tafazoli (1991) and Abo-El-Ez et al. (2002). They found that GA_3 increased yield. These data also are in agreement with Stopar and Zadravec (2001) on 'Gala' apple, as they reported that yield was decreased when trees were treated by BA. As for the effect of CPPU treatment, the present results are in the same line with Greene (1989) who noticed that foliar sprays of 10 and 100 ppm CPPU applied to 'McIntosh' apple trees at petal fall of the king blossom or 18 days later reduced crop load.

Physical Properties

1. Fruit Weight (g). Data presented in Table 2 show that average fruit weight of 'Samani' date palm was significantly higher in trees treated by sprays of GA_3 at 150 ppm, BA at 150 ppm and GA_3 at 100 ppm treatments recording 34.12, 32.36 and 31.47 g, respectively in the first season. However, in the 2nd season, treatments of GA_3 at 150 ppm, BA 150 ppm and CPPU at 20 ppm increased fruit weight significantly (33.42, 31.55 and 29.18 g, respectively). In this respect, the control, CPPU at 5 ppm and BA at 50 ppm treatments recorded the lowest significant average of fruit weight (15.31, 16.72 and 18.59 and 17.18, 18.63 and 20.52 g) in both seasons, respectively.

2. Fruit Flesh Weight. Data in Table 2 clearly indicate that GA_3 at 150 ppm, CPPU at 20 ppm and GA_3 at 100 ppm treatments gave significantly the highest fruit flesh weight (31.31, 29.36 and 28.85 g) in the first season and 30.89, 26.21 and 26.55 g in the second season. However, the control, CPPU at5 ppm and BA at 50 ppm gave the lowest fruit flesh weight (12.82, 14.82 and 16.46 g, respectively) during the 1st season and 14.62, 16.81 and 18.42 g, respectively during the 2nd season.

The results are in agreement with those reported by Maximos et al. (1980), Hussein et al. (1976), Mougheith and Hassaballa (1979), El-Nabawy et al. (1977), Abou-Aziz et al. (1982) and Abo-El-Ez et al. (2002). They found that different concentrations of GA_3 increased the fruit weight and flesh. Yuan RongCai and Greene (2000) on 'More-Spur McIntosh' apple, found that BA at 100 ppm increased fruit weight. Greene (1996) on 'McIntosh' apple recorded that CPPU treatment increased fruit weight by about 18-25%.

3. Fruit Length. Data in Table 2 indicate that fruit length was significantly affected by different treatments for 'Samani' in both seasons of study. In this respect, the best results were obtained from spraying GA_3 at 100 ppm, GA_3 at 150 ppm, BA at150 ppm, CPPU 10 ppm and CPPU at 20 ppm during the first season, while in the second season GA_3 at 150 ppm gave the longest fruits (5.82 cm). On the other hand, untreated trees and CPPU at 5 ppm gave the shortest fruits in both seasons.

4. Fruit Diameter. It is obvious from Table 2 that treatments of BA at 100 ppm and the control significantly produced the highest fruit diameter (3.9 and 3.8 cm), respectively in the first season, while in the second season date palm treated by GA_3 at 150 ppm, BA at 150 ppm, BA at 50 ppm, CPPU at 20 ppm, GA_3 at 100 ppm, CPPU at 10 ppm and GA_3 at 50 ppm produced the highest fruit diameter. In this respect, CPPU at 5 ppm treatment produced the lowest fruit diameter to a significant level (2.60 and 2.53 cm) in both seasons of the study, respectively. Similar observations were reported by many investigators: Mougheit and Hassaballa (1979), Maximos et al. (1980), El-Kassas (1986), Abou-Aziz et al. (1982), Hussein et al. (1976), Wazir (1985) and Abo-El-Ez et al. (2002). They found that fruit dimensions were increased when fruits received a gibberellic acid treatment. Also, the previous results agree with McLaughlin and Greene (1984) on 'Golden Delicious' apples, as they found that BA at 50 ppm increased fruit length and diameter. The obtained results also agree with those reported by Rao Jing Ping et al. (1999) on 'Fuji' apple, as they recorded that after full bloom application of CPPU at 5 or 10 ppm the fruit L/D ratio increased.

5. Fruit Size. 'Samani' was significantly affected by different treatments in both seasons. It was clearly noticed that GA_3 at 150 ppm, GA_3 at 100 ppm and CPPU at 20 ppm treatments gave the highest fruit size during the 1st season, while for the 2nd season data presented in Table 2 clearly indicate that fruit size of GA_3 at 150 ppm, BA at 150 ppm, GA_3 at 100 ppm, CPPU at 20 ppm and CPPU at 10 ppm treatments gave the highest fruit size. On the other hand, the control, CPPU at 5 ppm, BA at 50 ppm and GA_3 at 50 ppm gave the lowest fruit size in both seasons. These results are in agreement with those reported by Hussein et al. (1976) and El-Nabawy et al. (1977). They found that GA_3 treatment of 'Samani' dates resulted in greater fruit size.

Chemical Properties

1. Total Soluble Solids (TSS %). Data presented in Table 3 show that BA caused slightly increased total soluble solids during the two seasons. The lowest contents of total soluble solids in juice were in the treatments of CPPU and GA_3 at 50 or100 ppm. Similar results were reported by many authors such as El-Kassas (1986), Hussein et al. (1976) and Abo-El-Ez et al. (2002). They found that the total soluble solids decreased by treating with GA_3. Also, our results agree with those reported by Kang SungGeun et al. (1997) on 'Okitsu Early' and 'Miyagawa Early' apple trees, as they found that a post-bloom spray of BA on trees increased soluble solids concentration (SSC) in all cultivars. Sugiyama et al. (1993) observed that CPPU applied at 20 ppm to fruitlets of 'Golden Delicious' apple reduced the content of fruit soluble solids.

2. Total Acidity Percentage. Results in Table 3 indicate that total fruit acidity percentage was significantly affected by different treatments. BA treatments gave the lowest fruit acidity percentage. The control, GA_3 at 100 ppm and CPPU at 5 ppm treatments gave the highest fruit acidity percentage in both seasons. These results are in agreement with the trend reported by Abou-Aziz et al. (1982) on 'Sewy' dates.

3. Sugar Content. Data presented in Table 3 indicate that reducing sugar was significantly affected by different treatments in both seasons of study. Moreover, BA treatments gave the highest fruit content of reducing sugars in two seasons, while spraying with CPPU and GA_3 reduced reducing sugars during the two seasons of study.

Regarding non-reducing sugars, data in Table 3 indicated that fruit content at non-reducing sugars was affected significantly by different treatments. GA_3 at 150 ppm and BA at 100 ppm gave the highest fruit content of non-reducing sugars in the first season, on the other hand BA at 50 ppm and CPPU at 10 ppm gave the lowest contents of total non-reducing sugars in the first season, while in the second season GA_3 at 150 ppm and GA_3 at 100 ppm gave the highest value compared with other treatments.

As for total sugar content, results in Table 3 showed that fruit content of total sugars was significantly affected by different treatments of the same growth regulators in both seasons. BA at 50 or 150 ppm treatments increased total sugars in fruits in both seasons of study. CPPU at different concentrations and GA_3 at 100 ppm gave the lowest total sugars in fruits in the first season, while in the second season CPPU, the control and GA_3 at 50 or 100 ppm gave the lowest value. Similar results were reported by Hussein et al. (1976), Abou-Aziz (1982), El-Kassas (1986), Tartarini et al. (1993) and Abo-El-Ez et al. (2002).

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Tables

Table 1. Percentage retained fruits, bunch weight and yield of 'Samani' dates as affected by GA_3, BA and CPPU during the 2006 and 2007 seasons.

Treatment (ppm)	2006			2007		
	Fruit retained (%)	Bunch weight (kg)	Yield/tree (kg)	Fruit retained (%)	Bunch weight (kg)	Yield/tree (kg)
Control	48.52_{a}	20.72_{d}	124.3_{d}	42.56_{a}	19.25_{de}	118.6_{c}
GA_3 50	41.72_{b}	22.35_{c}	134.1_{c}	35.12_{c}	18.76_{ef}	112.6_{e}
GA_3 100	26.13_{e}	22.36_{c}	134.2_{c}	25.17_{f}	18.53_{f}	111.2_{e}
GA_3 150	28.62_{de}	24.81_{a}	148.9_{a}	23.52_{f}	21.80_{a}	130.8_{a}
BA 50	42.80_{b}	20.95_{d}	125.7_{d}	36.18_{bc}	19.36_{de}	116.2_{cd}
BA 100	32.80c	19.36_{e}	116.2_{e}	30.15_{de}	19.80_{cd}	118.8_{c}
BA 150	28.31_{de}	23.41_{b}	140.5_{b}	26.92_{ef}	20.40_{c}	122.4_{b}
CPPU 5	43.17_{b}	18.64_{ef}	111.8_{f}	38.63_{b}	17.21_{g}	103.3_{f}
CPPU 10	$26.93d_{e}$	18.36_{f}	110.2_{f}	31.42_{d}	18.86_{ef}	113.2_{de}
CPPU 20	30.18_{cd}	21.84_{c}	131.0_{c}	28.72_{de}	20.46_{b}	122.8_{b}

Means within each column with the same letter are not significant at 5% level.

Table 2. Physical properties of 'Samani' dates as affected by GA_3, BA and CPPU during the 2006 and 2007 seasons.

Treatment (ppm)	Fruit weight (g)		Flesh weight (g)		Fruit length (cm)		Fruit diameter (cm)		Fruit size (cm^3)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	15.31_{f}	17.18_{d}	12.82_{d}	14.62_{e}	4.40_{c}	3.98_{f}	3.80_{a}	3.02_{bc}	18.00_{d}	18.50_{d}
GA_3 50	20.59_{de}	19.36_{cd}	18.76_{c}	17.50_{de}	5.00_{b}	4.55_{de}	2.90_{cde}	3.18_{ab}	20.00_{d}	22.40_{cd}
GA_3 100	31.47_{abc}	29.40_{ab}	28.85_{ab}	26.55_{abc}	5.70_{a}	4.86_{bcd}	3.30_{b}	3.33_{ab}	36.00_{ab}	32.80_{a}
GA_3 150	34.12_{a}	33.42_{a}	31.31_{a}	30.89_{a}	5.70_{a}	5.82_{a}	3.40_{b}	3.55_{a}	40.00_{a}	36.00_{a}
BA 50	18.59_{def}	20.52_{cd}	16.46_{cd}	18.42_{de}	4.80_{b}	4.63_{cde}	2.80_{de}	3.40_{a}	20.00_{d}	22.00_{cd}
BA 100	21.70_{d}	23.72_{c}	19.65_{c}	21.64_{cd}	5.00_{b}	4.36_{ef}	3.90_{a}	2.79_{cd}	24.00_{cd}	25.50_{bc}
BA 150	32.36_{ab}	31.55_{ab}	25.43_{b}	29.22_{ab}	5.60_{a}	5.18_{b}	3.20_{bc}	3.50_{a}	32.00_{b}	35.00_{a}
CPPU 5	16.72_{ef}	18.63_{d}	14.82_{cd}	16.81_{de}	4.40_{c}	4.13_{f}	2.60_{e}	2.53_{d}	19.00_{d}	19.00_{d}
CPPU 10	27.19_{c}	28.46_{b}	25.05_{b}	25.93_{bc}	5.60_{a}	4.83_{bcd}	3.10_{bcd}	$3.22a_{b}$	30.00_{bc}	31.00_{ab}
CPPU 20	27.98_{bc}	29.18_{ab}	29.36_{ab}	26.21_{abc}	5.60_{a}	4.96_{bc}	3.40_{b}	3.36_{ab}	36.00_{ab}	32.00_{a}

Means within each column with the same letter are not significant at 5% level.

Table 3. Chemical properties of 'Samani' dates as affected by GA_3, BA and CPPU during the 2006 and 2007 seasons.

Treatment	TSS (%)		Acidity (%)		Reducing sugars (%)		Non-reducing sugars (%)		Total sugars (%)	
(ppm)	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	21.82_{b}	20.50_{cd}	0.215_{ab}	0.218_{ab}	56.81_{bc}	55.42_{bc}	6.72_{b}	8.88_{c}	63.53_{cd}	64.30_{cd}
GA_3 50	20.56_{bc}	20.16_{de}	0.218_{ab}	0.203_{b}	53.82_{cde}	56.18_{b}	7.83_{b}	8.63_{c}	61.65_{def}	64.81_{cd}
GA_3 100	20.18_{bc}	18.83_{de}	0.246_{a}	0.210_{ab}	51.48_{de}	53.43_{bc}	6.99_{b}	11.40_{ab}	58.47_{fg}	64.83_{cd}
GA_3 150	22.05_{b}	22.62_{bc}	0.196_{b}	0.206_{ab}	53.14_{de}	54.26_{bc}	11.27_{a}	12.27_{a}	64.41_{bcd}	66.53_{bc}
BA 50	25.30_{a}	24.23_{ab}	0.183_{b}	0.197_{b}	62.82_{a}	61.45_{a}	4.60_{c}	8.17_{c}	67.42_{ab}	69.62_{a}
BA 100	24.51_{a}	25.00_{a}	0.189_{b}	0.193_{b}	54.73_{cd}	60.72_{a}	10.45_{a}	7.38_{c}	65.18_{bc}	68.10_{ab}
BA 150	26.48_{a}	25.80_{a}	0.176_{b}	0.187_{b}	61.36_{ab}	62.23_{a}	8.27_{b}	8.69_{c}	69.63_{a}	70.92_{a}
CPPU 5	19.47_{c}	18.15_{e}	0.252_{a}	0.238_{a}	50.82_{e}	52.66_{c}	6.66_{b}	9.44_{bc}	57.48_{g}	62.10_{d}
CPPU 10	19.41_{c}	20.41_{cd}	0.248_{a}	$0.216a_{b}$	53.32_{cde}	$54.42b_{c}$	6.31_{bc}	9.45_{bc}	59.63_{efg}	63.87_{cd}
CPPU 20	$21.3b_{c}$	20.89_{cd}	0.203_{b}	$0.221a_{b}$	54.83_{cd}	$55.16b_{c}$	8.00_{b}	7.77_{c}	62.83_{cde}	62.93_{d}

Means within each column with the same letter are not significant at 5% level.

Effect of Offshoot Weight on Establishment and Vegetative Growth in 'Sayar' Date Palm

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Keywords: irrigation, height, leaf, leaflet, sucker

Abstract

Date palm (*Phoenix dactylifera* L.) is propagated traditionally by offshoots which are produced in a limited number in a palm tree. Success of establishment of date palm offshoots is different between date palm cultivars. It depends on some factors such as offshoot weight, age and root system. This study was carried out in a randomized complete block design with three treatments of offshoot weight and twelve replications. Weight of offshoots was 10, 12 and 16 kg. The offshoot growth characteristics such as establishment percent, plant height, number of leaves, leaf length and width, number of leaflets, leaflet length and width, trunk diameter and canopy coverage were measured. The results showed that offshoot weight treatments had no significant effect on establishment of date palm offshoot, but there was significant difference between number of leaves, number of leaflets and leaflet width. The highest number of leaves, number of leaflets and leaflet width is resulted from 16 kg treatment. There was significant difference between this treatment with 10 and 12 kg treatments. But there was no significant difference between 10 and 12 kg offshoots.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the important and strategic fruits in Iran. The area under date palm cultivation and annual date production in Iran are 244,429 ha and 1,006,406 tons, respectively. Among different date palm cultivars, 'Sayer' date palm is one of the most important cultivars in Iran. Date palm is propagated traditionally by offshoots which are produced in a limited number for a certain period in the life of a young palm tree. This method of propagation is limited in its scope because the low percentage of offshoots survival, due to poor root formation. The rate of rooting varies with cultivar. Meanwhile, it is low in small offshoots and increases with increasing size. Date palm trees during the initial months of establishment require frequent irrigation particularly under dry conditions. It ensures better survival, quick growth and precocious bearing. Prolonged water stress conditions during this period may even result in the drying of the offshoots. The upper surface of the soil must be kept moist for the survival of young date palm offshoots.

Saaidi et al. (1989) studied some factors affecting rooting of date palm offshoots. 78% of 'Bou Slirene' offshoots rooted whereas with 'Tadment', 'Iklane' and 'Bou Stammi Noir' the offshoots percentages were 64, 53 and 31, respectively. There was little difference in the rooting ability of offshoots weighing 3-5 kg, 5-7 kg or 7-10 kg. Irrigation was an important factor.

Al-Ghamdi (1988) studied a new method of injecting different concentrations of IBA in three different sizes of 'Khalas', 'Ruziz' and 'Shishi'. Cultivars and offshoots sizes show a highly different response in root formation.

In India, offshoots should be irrigated daily up to 10 days after plantation and at alternate days during the next two months (Chandra and Gupta, 1995). Thereafter, irrigation should be given at least once a week during winter and twice a week during summer.

Broschat (1994) determined the effects of leaf removal, leaf tying, and overhead irrigation during transplanting using mature pygmy date palms (*Phoenix roebelenii*). In

one experiment palms had all, 2/3, or none of their leaves removed and were irrigated overhead or on the soil surface only. In the second experiment, palms had 2/3 of their lower leaves removed and those remaining were either tied up into a bundle or left untied. Half of the palms in each experiment were irrigated daily while the other half was subjected to water stress cycles. Results showed that leaf tying had no positive effect on the palms, but when coupled with overhead irrigation, was responsible for fungal infections in the crowns of some palms. With regular irrigation, palm quality and root growth were increased as the number of leaves retained was increased, but under water stress conditions, the reverse was true. Overhead irrigation had no positive effect on transplanted palms.

Hodel et al. (2003) investigated the effect of removing leaves and tying up leaves when transplanting palms. Several combinations of leaf tie up and/or removal during transplanting did not affect establishment and survival of small, juvenile Canary Island date palms (*Phoenix canariensis*) and queen palms (*Syagrus romanzoffiana*). For both species, complete leaf removal resulted in the least amount of new leaf and root growth. None of the combinations resulted in more new leaf or root growth or reduced leaf transpiration rates and leaf water potential than no leaf removal and no tie up. Leaf tie and/or removal appear to offer no benefit to transplanted, juvenile palms provided the root ball and backfill are kept moist.

Lahav (1992) showed that irrigation had a marked effect on the potassium content of the third leaf of the banana sucker. In trials carried out in 1994 and 1995, 2- and 3-year-old 'Haden' mango trees were cultivated at 4 different drought stress levels (Pire et al., 1995). Watering frequency varied from every 2-3 days in the wettest treatment to every 14-20 days in the driest. After 5 weeks, all plants were irrigated on alternate days with 3 L of water each. Substrate humidity, plant water status, leaf area, plant height and gas exchange were measured just before restarting watering, while vegetative and floral shoot production were monitored during the next 3 weeks or so. In the 2-year-old plants there was no flowering even 20 days after watering restarted, possibly due to juvenility, but buds were formed, increasing in number from 0.13 to 0.62 per branch as the level of drought stress decreased. In the 3-year-old plants, the same tendencies were found with respect to the formation of buds. Flowering occurred in 25% of the plants, but without a definite tendency with respect to water stress level. The results seem to confirm the fact that water stress is not a mechanism encouraging the formation of mango flowering but it does affect vegetative growth. A field experiment was conducted to evaluate the effect of different irrigation regimes on the vegetative development and precocity of young dwarf coconut palms (Miranda et al., 1999). Irrigation treatments 1, 2, and 3 were applied when the accumulated Class A pan evaporation reached 10, 30 and 50 mm, respectively. Irrigation intervals varied from 1 to 2 days in treatment 1, from 3 to 4 days in treatment 2, and from 5 to 7 days in treatment 3. The values for number of leaflets on leaf 3 and collar girth were highest in treatment 1. No significant differences between treatments were observed for number of leaves per palm and number of leaves produced. Harvest started when plants were 28 months old. During the third year, an average yield of 118 fruits per plant was obtained, and no significant differences were found among the treatments.

MATERIALS AND METHODS

This study has been conducted in the Date Palm and Tropical Fruits Research Institute, Germplasm Collection, Ahvaz city (Iran), 22.5 m a.s.l., on 'Sayar' offshoots (Table 1). This experiment has been carried out using a Randomized Complete Block Design (RCBD) with three treatments of offshoot weight and twelve replications in two years. The offshoots were cultured in every year. Weight of offshoots was 10, 12 and 16 kg. Soil samples were obtained from different depths (0-30, 30-60, 60-90 cm). Then soil samples and an irrigation water sample were sent to the laboratory for analyses (Tables 2 and 3). The amount of required irrigation water for the treatments due to amount of evaporation from class A pan has been estimated with the FAO pan method. Crop evapotranspiration (ETc) with FAO pan method is equal to:

$$ETc = Kc.Kp.Ep \quad (1)$$

where Ep=amount evaporation from pan (mm), Kp=pan coefficient and Kc=crop coefficient. The amount of Kp and Kc was estimated based on FAO tables (Allen et al., 1998). Other agricultural practices were done similarly to all treatments. The offshoot growth characteristics such as establishment percent, plant height, number of leaves, leaf length and width, number of leaflets, leaflet length and width, trunk diameter and canopy coverage were measured in every year.

RESULTS AND DISCUSSION

The averages of offshoot establishment percent, number of leaves, leaf length and width in different treatments are shown in Table 4. Results showed that date palm offshoot weight treatments had no significant effect on offshoot establishment and leaf length and width, but there was significant difference in number of offshoot leaves. The offshoot establishment was 100% in all treatments. Saaidi et al. (1989) reported that irrigation was an important factor affecting rooting of date palm offshoots. The maximum number of leaves resulted from 16 kg offshoots. There was no significant difference between 10 and 12 kg offshoots. The averages of increasing plant height, number of leaflets, leaflet length and width in different treatments are shown in Table 5. Results showed that offshoot weight had significant effect on number of leaflets and leaflet width, but there was no significant difference in increasing plant height and leaflet length. Maximum and minimum of leaflet number was 207 and 103, respectively. The highest number of leaflets resulted from 16 kg offshoots. There was significant difference between this treatment with 10 and 12 kg treatments. But there was no significant difference between 10 and 12 kg offshoots. Maximum and minimum of leaflet width was 1.3 and 1.0 cm, respectively. The highest width of leaflets resulted from 16 kg offshoot. There was significant difference between 16 kg treatment and other treatments. There was no significant difference between 10 and 12 kg offshoots.

Offshoot size may be important for resprouting of cut roots. Al-Mana et al. (1996), Reuveni et al. (1972) and Reuveni and Adato (1974) concluded that the larger offshoots have more stored carbohydrates for energy for root growth, higher levels of root-promoting substances, and lower levels of root-inhibiting substances than smaller offshoots. Hodel and Pittenger (2003) reported that offshoots with at least 30 cm leaf extension and 29 existing roots established most successfully. Those with at least 22 green leaves when removed from the mother plant generally grew more roots. Although some leaf extension occurred even before the offshoots were replanted, offshoots with at least 24 cm leaf extension are probably successfully established.

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Tables

Table 1. Meteorology data mean of Ahvaz city in 2007 and 2008 years.

Month	Temperature (°C)		R. humidity (%)		Precipitation (mm)	Evaporation (mm)
	Min	Max	Min	Max		
January	3.8	16.8	36.4	81.6	15.7	59.1
February	7.9	19.6	37.4	81.4	20.1	75.3
March	10.4	25.9	21.0	70.4	3.6	137.8
April	16.0	31.4	22.4	72.0	21.2	243.6
May	21.8	39.0	12.5	52.5	2.9	378.8
June	22.5	42.1	6.3	40.3	0.2	637.5
July	27.2	46.0	7.4	36.8	0.0	700.8
August	28.1	47.5	8.6	45.4	0.0	553.9
September	26.1	45.3	11.7	53.3	1.8	388.8
October	19.0	38.5	12.1	47.3	0.0	289.5
November	18.6	30.1	24.7	54.9	10.0	158.6
December	8.7	21.6	37.5	82.5	27.3	86.9

Table 2. Physical and chemical characteristics of soil.

Soil depth (cm)	Soil texture	K (ppm)	P (ppm)	OC (%)	pH	Mg (meq/L)	Ca (meq/L)	Na (meq/L)	EX (dS/m)	SAR
0-30	Clay loam	170.0	12.0	0.59	7.8	16.5	7.2	12.5	4.9	3.5
30-60	Clay loam	252.1	10.0	0.43	7.7	24.4	4.8	9.1	4.9	2.4
60-90	Clay loam	285.4	10.1	0.36	7.2	23.3	4.9	9.8	5.2	2.6

Table 3. Analysis results of irrigation water.

Cation (meq/L)			Anion (meq/L)			pH	EC (dS/m)	SAR
Ca^{2+}	Mg^{2+}	Na^{+}	HCO_3^{-}	Cl^{-}	SO_4^{2-}			
4.0	12.0	9.9	-	-	3.0	8.0	2.3	3.5

Table 4. Offshoot establishment, number of leaves, leaf length and width.[1]

Offshoot weight (kg)	Establishment (%)	Number of leaves	Leaf length (cm)	Leaf width (cm)
10	100 a	3 b	98.7 a	30.9 a
12	100 a	3 b	105.7 a	34.6 a
16	100 a	4 a	121.6 a	44.5 a

[1] Means of each column separation at 5% level (DMRT).

Table 5. Offshoot increasing height, number of leaflet, leaflet length and width.[1]

Offshoot weight (kg)	Height (%)	Number of leaflets	Leaflet length (cm)	Leaflet width (cm)
10	40.1 a	103 b	26.9 a	1.0 b
12	43.3 a	119 b	30.5 a	1.1 b
16	51.3 a	207 a	32.5 a	1.3 a

[1] Means of each column separation at 5% level (DMRT).

Response of Date Palm Trees to Different Nitrogen and Potassium Application Rates

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Keywords: date palm, nitrogen, potassium, yield, fruit quality, leaf mineral content

Abstract

The present study was carried out during the 2007 and 2008 growing seasons on 'Zaghloul' and 'Hallway' date palms grown in clay soil in a private orchard located at El-Tarh region, El-Beheira governorate, in order to study the effect of NK application on palm yield, leaf mineral content and fruit quality. Both fertilizers were applied at the rate of 0.0 (N_0 or K_0), 500 (N_1 or K_1) and 1000 (N_2 or K_2) g per palm. The data of both cultivars showed that the N and K fertilization resulted in a significant higher yield per palm than those of the control. The application of 500 and 1000 g K_2O significantly increased fruit weight and length. In addition, K fertilization increased fruit TSS whereas it decreased the tannins contents. Both fruit reducing and total sugars were significantly increased by potassium application. Potassium fertilization increased leaf N, K, Fe and Zn and decreased Ca, Mg and Mn contents, with the highest content by the higher rate. Nitrogen fertilization increased fruit tannins and decreased fruit TSS contents. 'Zaghloul' fruits had significantly higher fruit reducing and total sugars than 'Hallway' fruits. The percentage of leaf N, Ca and Mg content was increased with nitrogen application and with increasing the nitrogen rate. Moreover, leaf nitrogen content of 'Zaghloul' palms was significantly higher than that in 'Hallway'.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees in the world, known as the tree of life because of its resilience, its need for limited water inputs, its long term productivity and its multiple purpose qualities. In Egypt dates are an important traditional crop. According to FAO (2009) Egypt is considered the first country of the top ten date producers (1,130,000 tons). In addition, 'Zaghloul', 'Samany', 'Hallway' and 'Hayany' seem to be the most predominant soft date palm cultivars grown in Egypt. Their fruits are highly recommended and demanded in the local and international market.

Date palm yield and fruit quality are mostly dependent on cultivar, nutrition and water relations. The efficient use of fertilizers to increase crop yield is an important goal in all agricultural systems, thus, the adaptation of a proper fertilization program in order to maximize nutrient uptake and minimize fertilizer application has been and will continue to be an ambitious pursuit for both researchers and growers as well. Available information about the nutritional requirements of the different date palm cultivars are generally lacking. Investigations have been made on some date cultivars in order to determine the nutrition regimes and fertilizer necessity applied to date palms, and their influence on palm growth, productivity and fruit quality (El-Hammady et al., 1994; Kassem et al., 1997). A rate of nitrogen application ranging from 800 to 1100 g N per palm was reported (Sinclair et al., 1981; Bliss and Mathez, 1983; Karami, 2007).

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Moreover, Bamiftah (2000) recommended an amount of 2 or 3 kg of potassium sulphate/palm/year for high yield and fruit quality.

Therefore, the present investigation was carried out in order to study the effect of nitrogen and potassium fertilization rates on yield, fruit quality and leaf mineral content of 'Zaghloul' and 'Hallway' date palm cultivars grown in a clay soil.

MATERIALS AND METHODS

Plant Material

The present study was conducted during the 2007 and 2008 growing seasons on 28-year-old 'Zaghloul' and 'Hallway' date palm cultivars grown in a clay soil and spaced 10×10 m apart in a private orchard located at El-Tarh region, El-Beheira governorate, in order to study the effect of nitrogen; as ammonium sulphate and potassium; as potassium sulphate on fruit yield, fruit quality and leaf mineral content. The orchard was irrigated with Nile water and fertilized only with organic manure (1.5% N) at the rate of 20 kg/tree in December of each season. Soil analysis according to the method of Chapman and Pratt (1961) is presented in Table 1. 27 female palms of each cultivar were selected as uniform as possible in growth, vigor, height, pollen source and age, and were subjected to the normal cultural practices usually carried out for date palms. The leaf/bunch ratio was adjusted, in both years, by the end of the blooming season to meet the value of 10:1 and 8:1 for all experimental palms of 'Zaghloul' and 'Hallway', respectively.

Experimental Treatment

Nitrogen fertilizer as ammonium sulphate (21.5% N) was applied at three different rates, i.e., N_0=0.0, N_1=500 and N_2=1000 g N/palm, respectively. Potash fertilizer as potassium sulphate (48% K_2O), was applied at three different rates, i.e., K_0=0.0, K_1=500 and K_2=1000 g K_2O/palm, respectively. Nitrogen fertilizer was divided into three equal doses and added to the trees in March, May and July of both experimental years. Potassium fertilizer was divided into two equal doses and added in March and July. Both fertilizers were added either alone or in combinations as a broadcast on the soil surface, 1.5 m apart from the palm trunk and palms were irrigated immediately after fertilizers application. 18 fertilizing treatments, representing all the possible combinations of the three levels of nitrogen, three levels of potassium and two date palm cultivars (3×3×2=18 treatments), and 27 trees for each cultivar (3 repl./treat. × 3 N levels × 3 K levels=27 trees) were used in this study.

Yield and Fruit Quality Determination

The yield of each studied palm was recorded in kg at harvest time (mid-November) of both seasons. In order to determine fruit quality characteristics a sample of 30 mature fruits was taken at random from four different bunches of each replicate (palm) in both seasons. Fruit weight (g) and length (cm) were determined in all fruit samples. The percentage of total soluble solids (TSS) in fruit juice was determined by a hand refractometer. Tannins were determined by the method of Swain and Hillis (1959). Reducing, non-reducing and total sugars were determined in fresh fruit samples according to the method described by Malik and Singh (1980).

Leaf Mineral Content

At the beginning of November of both seasons; three consecutive leaves (located just below the fruiting leaf zone and about two years old) were chosen at random from each tree for a given cultivar. Five median pinnate were removed from each side of the mid-point of the laminar-pinnae-bearing portion of the rachis, making a total of 30 pinnae per leaf sample, as described by Reuther (1948). The pinnae samples were washed with tap water, rinsed two times with distilled water and oven dried at 65-70°C to a constant weight. The dried pinnae tissues were ground and digested with sulphuric acid and hydrogen peroxide, as outlined by Evanhuis and DeWaard (1980). Suitable aliquots were

then taken for the determination of mineral elements. Nitrogen and phosphorus were determined calorimetrically according to Evanhuis (1976) and Murphy and Riley (1962), respectively. Potassium was determined by flame photometer. Calcium, magnesium, iron, manganese and zinc were determined by using a Perkin Elmer atomic absorption spectrophotometer.

Statistical Analysis

All data were tested for treatments effects on analyzed parameters by analysis of variance ANOVA using the Statistical Analysis System (SAS Institute, 1996).

RESULTS AND DISCUSSION

Leaf Mineral Contents

Regardless of potassium fertilizer and date cultivar, the results of both seasons presented in Table 2 reveal that in general leaf N, Ca and Mg contents increased significantly with increasing nitrogen level. These results are supported by those reported by Bacha and Abou-Hassan (1983), El-Hammady et al. (1987) and Kassem et al. (1997). In addition, the N_2 treatment caused a marked reduction in the leaf P content during both seasons. Bacha and Abou- Hassan (1983) and Aly (1993) found that the N fertilization did not affect leaf P content, whereas, El-Hammady et al. (1987) reported that the N fertilization increased leaf P content. The data also showed that N fertilization treatments did not significantly affect leaf K, Na and Mn contents in both seasons. These results are in agreement with those reported by Bacha and Abou-Hassan (1983) and Aly (1993). On the other hand, El-Hammady et al. (1987) found that the N fertilization increased pinnae K, but decreased pinnae Na content. The leaf Fe and Zn contents, during both seasons, significantly decreased with increasing N level. These results partially agreed with those reported by Aly (1993) and Kassem et al. (1997) who found that the lowest Fe content was observed with N fertilization.

As for the effect of potassium fertilization the data presented in Table 2 showed that in both seasons, leaf N, K, Fe and Zn contents tended to increase with increasing the rate of K fertilization. Whereas, K fertilization did not greatly affect leaf P content. Moreover, K application tended to decrease leaf Mg, Ca and Mn contents as compared with the control. These findings supported those previously reported by Montasser et al. (1994) and Kassem et al. (1997).

Concerning the differences between the two date palm cultivars the results presented in Table 2 indicated that the 'Zaghloul' pinnae N content was significantly higher than that in 'Hallway' during both seasons, which had higher pinnae Fe and Zn during both seasons. The results also revealed that the differences were not high enough to be significant between the two date palm cultivars in leaf P, K, Ca, Mg and Mn contents. The fact that the date cultivars vary in their pinnae mineral composition, was reported by Kassem et al. (1997) and Ibrahim and Sinbel (1989).

Yield

Regardless of potassium fertilization and date cultivar, the obtained results in Table 3 indicate that in both seasons the palms that received different nitrogen fertilization rates had a significantly higher yield than those of the control. No significant differences were found between the N_1 and N_2 in the first season whereas; in the second season the N_2 was higher than N_1. A marked improvement in the yield of several date cultivars by nitrogen fertilization was also recorded. Hussein and Hussein (1983) reported that 750 g N/palm increased yield of 'Sakkoti' dates. In the meantime, El-Hammady et al. (1987) found that 'Seewy' palms that received 500 or 750 g N/palm/year yielded higher than those that received 1000 g N/palm. Karami (2007) observed that application of 800 g nitrogen per palm to 'Mordaseng' dates caused the highest yield production.

Regardless of nitrogen fertilization and date cultivar, the data presented in Table 3 show that potassium fertilization increased date yield during both seasons. In the first season

the K_2 only caused a significant increase whereas, in the second season both K_1 and K_2 increased the yield, with no significant difference obtained between both levels. This increment in fruit yield was also reported by numerous investigations working on different dates (Abdalla et al., 1987; El-Hammady et al., 1994; Kassem et al., 1997). They found that the yield was significantly increased by potassium fertilization. On the other hand, Bacha and Abou-Hassan (1983) found that potassium fertilization had no effect on yield.

Regardless of nitrogen and potassium fertilization, the data in Table 3 indicate that, in the first season, the yield of 'Zaghloul' was significantly higher than that of 'Hallway'.

Fruit Physical Properties

The results indicated in Table 3 showe that, regardless of potassium fertilization and date cultivar, the 500 g nitrogen/date increased fruit weight and length as compared with the control in the second season. Whereas, 1000 g N did not exhibit any favorable effect. Similarly, El-Hammady et al. (1987) reported that increasing the nitrogen rate to 1000 g did not affect fruit diameter and length of 'Seewy' dates.

Regardless of nitrogen fertilization and the date cultivars, the data presented in Table 3 show that both rates of potassium fertilization significantly increased the fruit weight and length of date palm during both seasons, as compared with the control. The K_2 resulted in the highest effect. These results agreed with those reported by Abdalla et al. (1987), El-Hammady et al. (1994) and Kassem et al. (1997). However, Bacha and Abou-Hassan (1983) stated that increasing the potassium rate had no effect on fruit length.

Regardless of nitrogen and potassium fertilization, the data presented in Table 3 show that, during both seasons, there was a significant increase in fruit weight and length for 'Zaghloul', compared with 'Hallway'. A higher fruit weight of 'Zaghloul' dates than 'Hayany' was recorded by Kassem et al. (1997).

Fruit Chemical Properties

1. Fruit TSS and Sugars. Data in Table 3 indicate that nitrogen fertilization tended to decrease the fruit TSS, reducing and total sugars contents during both seasons specially, the N_2 application. Similarly, Hussein and Hussein (1983) and Kassem et al. (1997) reported that the date fruit TSS decreased with higher nitrogen application. On the other hand, El-Hammady et al. (1987) found that the nitrogen fertilization did not affect fruit TSS.

As for the effect of potassium fertilization rates only, the data of both seasons showed that fruit TSS, reducing and total sugars contents were significantly increased in comparison with the control, while the non-reducing sugars had no effect (Table 3). Similar findings were reported by El-Hammady et al. (1994), Kassem et al. (1997) and Al-Kharusi et al. (2009). On the contrary, Bacha and Abou-Hassan (1983) and Abdalla et al. (1987) found no effect of potassium fertilization on date fruit sugars content.

The data of both seasons indicated that the fruits of 'Zaghloul' contained significantly higher fruit TSS, reducing and total sugars contents than 'Hallway', while the fruit non-reducing sugars did not vary between the two studied date cultivars (Table 3). These results are in general agreement with those of Vandercook et al. (1980), Ibrahim and Sinbel (1989) and Kassem et al. (1997) who all stated that sugars content was cultivar dependent.

2. Tannins. With regard to nitrogen application only, fruit tannins content tended to increase with nitrogen fertilization in both seasons as compared with the control (Table 3). Similarly, Aly (1993) and Kassem et al. (1997) found that the nitrogen fertilization increased date fruit tannins. In contrast, the potassium fertilization tended to decrease fruit tannins. This reduction was significant in the second season only by the K_2 level (Table 3). Likewise, El-Hammady et al. (1994) and Kassem et al. (1997) reported that potassium fertilization reduced date fruit tannins content. In addition, results of both seasons indicated that the fruit of 'Hallway' contained significantly higher tannins than 'Zaghloul' (Table 4).

CONCLUSION

From the above mentioned we might conclude that the application of 500 g of both nitrogen and potassium fertilization per palm could be suitable for increasing palm yield and improving date quality of 'Zaghloul' and 'Hallway'.

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Tables

Table 1. Soil analysis of the experimental orchard.

Parameters	EC (mmhos/cm)	pH	$CaCO_3$ (%)	Organic matter (%)	Texture	Ca^{++} (meq/L)	Mg^{++} (meq/L)	K^{+} (meq/L)	P (meq/L)
Value	1.12	8.20	8.04	0.72	Clay	6.31	3.56	0.18	61

Table 2. Mean effects of NK fertilization and cultivar on leaf macro (%) and micro (ppm) nutrients content in the 2007 and 2008 seasons.

Mineral	N^z			K^y			Cultivar^x	
contents	N_0	N_1	N_2	K_0	K_1	K_2	Zaghloul	Hallway
					2007			
N %	2.15b	2.35ab	2.52a	2.13b	2.50a	2.40a	2.44a	2.23b
P %	0.32a	0.27ab	0.23b	0.29a	0.26a	0.27a	0.26a	0.29a
K	0.96a	0.88a	0.96a	0.88b	0.92b	1.01a	0.94a	0.92a
Ca	1.23b	1.31a	1.32a	1.25b	1.28ab	1.33a	1.30a	1.27a
Mg	0.43b	0.54a	0.56a	0.47b	0.58b	0.65a	0.53a	0.49a
Fe	134a	123ab	116b	119b	120b	137a	117a	132b
Zn	74a	79a	56b	63b	70ab	77a	64a	76b
Mn	53a	52a	48a	58a	50ab	45b	54a	49a
					2008			
N %	2.26b	2.53a	2.62a	2.33b	2.48ab	2.60a	2.55a	2.39b
P %	0.37a	0.29ab	0.24b	0.30a	0.28a	0.32a	0.28a	0.32a
K	0.86a	0.80a	0.82a	0.70c	0.81b	0.91a	0.84a	0.82a
Ca	1.26b	1.34a	1.32a	1.35a	1.31a	1.26b	1.37a	1.24a
Mg	0.43b	0.52ab	0.58a	0.62a	0.55ab	0.53b	0.60a	0.54a
Fe	143a	130ab	123b	120c	122bc	144a	122a	142b
Zn	68a	52b	56b	52b	60a	64a	55a	62b
Mn	56a	61a	54a	63a	59ab	49b	54a	60a

z, y x: Values within a row with the same letter are not significantly different ($p<0.05$).

Table 3. Mean effect of NK fertilization and cultivar on yield and fruit quality during the 2007 and 2008 seasons.

Mineral contents	N[z]			K[y]			Cultivar[x]	
	N_0	N_1	N_2	K_0	K_1	K_2	Zaghloul	Hallway
					2007			
Yield (kg/tree)	173b	218a	227a	190b	200b	228a	217a	195b
Fruit weight (g)	24.7a	26.0a	25.3a	20.7c	26.7b	28.6a	28.2a	22.5b
Fruit length (cm)	4.64a	5.10a	4.42a	4.11	4.74	5. 2	5.08	4.36
TSS (%)	33.5a	32.4ab	31.3b	30.6b	32.8a	33.8a	33.9a	30.9b
Non-reducing sugars (%)	5.52a	5.56a	4.97a	4.73a	4.19a	6.13a	5.57a	5.13a
Reducing sugars (%)	27.2a	26.5a	24.4b	23.9c	25.8b	28.3a	26.8a	25.2b
Total sugars (%)	32.7a	32.0a	29.1b	28.6c	31.0b	34.4a	32.3a	30.4b
Tannins (%)	0.68b	1.06ab	1.32a	1.26a	0.97a	0.83a	0.75b	1.31a
					2008			
Yield (kg/tree)	153c	178b	197a	156b	182a	190a	172a	181a
Fruit weight (g)	26.1b	28.3a	26.8b	24.8c	26.5b	29.9a	30.6a	23.6b
Fruit length (cm)	4.77	5.60	5.07	4.36	5.14	5.93	5.56	4.73
TSS (%)	28.2a	27.2ab	25.9b	25.1b	27.7a	28.5a	28.9a	25.3b
Non-reducing sugars (%)	5.52a	5.56a	4.97a	4.56a	5.54a	5.55a	5.42a	5.01a
Reducing sugars (%)	26.4a	25.4a	22.5b	24.9b	26.3ab	28.0a	27.1a	25.7b
Total sugars (%)	31.9a	30.6b	27.3c	29.4b	31.9a	33.5a	32.5a	30.7b
Tannins (%)	0.80b	1.15ab	1.27a	1.45a	0.93b	0.85b	0.68b	1.46a

z, y x: Values within a row with same letter are not significantly different ($p<0.05$).

Interaction of Sulfur and Magnesium on Palm Oil Seed

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Keywords: magnesium, sulfur, oil, seed, palm

Abstract

Sulfur and magnesium are two macronutrient elements for plants and other organisms. Sulfur has different roles in plants such as; amino acids production and adjustment of growth controllers the same as thiamine, resistance of plants in cold because of sulfhydryl, increasing of oil in oleaginous plants. Also the roles of magnesium in plants are different; it is activator of numerous enzymes especially active of phosphorus carriers, in the metabolism of hydrocarbon, particularly in the citric acid cycle and plants respiration, also magnesium with sulfur has an effect in the production of oil in plants. In this study for examination of sulfur and magnesium on oil seed of *Phoenix dactylifera* 'Shahani' four levels of magnesium were used (0, 40, 80, 120 mg Mg kg^{-1} soil) by $Mg(NO_3)_2$ and four levels of sulfur (0, 40, 80, 120 mg S kg^{-1} soil) by $(NH_4)_2SO_4$. These treatments were added to the soil in the first half of February and irrigation was done by drip method. In end of September harvest took place and seeds were separated from fruits and ground. Then oil was separated from other seeds' parts by boiling water. The results of this experiment are shown; increasing the magnesium application had a positive significant effect on seed oil. A level of 120 mg of magnesium had a positive significant effect on seed oil compared to the control and other magnesium levels and this level increased seed oil by 15% related to the control. Levels of 80 and 120 mg sulfur had no significant effect with other, but they had a significant effect with other levels, especially with the control, these levels increased seed oil by 12% related to the control. When these elements were used together, with a higher application of these elements seed oil was increased. The highest amount of seed oil was obtained with the treatment with 120 mg magnesium and 80 mg sulfur; it showed a positive significant increase compared to other treatments and increased the seed oil by 15% related to the control. The statistical design applied in this study was a Complete Randomized Design (CRD) with sixteen treatments and three replications. Means' comparisons are compared by Duncan's test. For statistical analysis of data the computer software MSTATC was used.

INTRODUCTION

Dates are plants that historically have always enjoyed the attention and respect of human beings. Many properties of this plant are similar to human. All parts of this plant are used by humans. This plant as a food source is filled with energy and minerals, it provides the seeds for the oil industry and its trunk is used in building. This plant has many species of fruit, but few species are involved in human feeding. Nutrients are environmental factors effective in the growth of plants as fertilizer to the plants is provided and they can have a large role in the quantity and quality of agricultural products. Nutrients have any specific role, some of these elements play a role in growth and part of the elements have a role in flowering and procreation. Also some improve the quality and some increase the quantity of agricultural products. In this study, the interaction effect of sulfur and magnesium in increasing the amount of seed oil palm cultivars (*Phoenix dactylifera* 'Shahani') was analyzed.

Sulfur has different roles in plant such as; amino acids production and adjustment of growth controllers same as thiamine, resistance of plants in cold because of sulfhydryl,

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increasing of oil in oleaginous plants. Also, the roles of magnesium in plants are different; it is activator of numerous enzymes, especially active for phosphorus carriers, in the metabolism of hydrocarbon, particularly the citric acid cycle and plants respiration. Also magnesium with sulfur has an effect in the production of oil in plants. Some elements as potassium and manganese have a negative effect on the uptake of magnesium so it can have a negative influence on chlorophyll making and oil productivity in olive. The application of olive oil wastewater tends to reduce the concentration of Mg in the plant, similarly to the effect of adding mineral potassium fertilizer. An enhancement of Mn availability takes place in the soil amended with olive oil wastewater, which on occasion has produced Mn concentrations in plants that could be considered phytotoxic or at least excessive. After harvesting, we observed an increase in the amount of exchangeable K in soil with added industrial wastewater. However, these increases are lower than those in soil treated with mineral potassium fertilizer. The levels of exchangeable, carbonate-bound, organic-bound and residual Mg in soil were higher in treatments incorporating olive oil wastewater than in those with added mineral K, with the opposite tendency occurring in the amount of Fe-Mn oxides-bound Mg in soil (Gallardo, 2000). Although its economic products (palm oil and palm kernel oil) contain mainly carbon (C), hydrogen (H) and oxygen (O), the oil palm has a large requirement for nutrients that is only surpassed by a few crops, such as banana (Soh, 1997). Whilst the first commercial oil palms were planted on fertile coastal clay soils in Malaysia, liparitic soils in north Sumatra and volcanic soils in west Sumatra, most oil palms are now planted on poor fertility status 'inland' or 'upland' soils on the islands of Borneo and Sumatra and in Thailand. Nutrient losses due to surface erosion and runoff are generally greater in these countries due to the pre-dominantly hilly terrain, fragile soil structure and high rainfall. Thus, mineral elements as Mg and S are of great importance to supplement the poor indigenous soil nutrient supply, and large yield responses have been demonstrated in many fertilizer experiments carried out in the region (Foster and Goh, 1997). The oil palm is predominantly cultivated on tropical soils that belong mainly to the soil orders Ultisol, Oxisol and Inceptisol. These soils are highly acidic and have low buffering capacities. Consequently, fertilizers are essential for economic production as attested by ample field experiments and growth in fertilizer usage in the oil palm sector. For good yields to be sustained, fertilizer inputs are necessary and typically constitute 40-50% of total field up keep cost. With palm oil projected to grow to 35 million tons by 2020, the expansion in fertilizer requirements is assured and this makes pleasant news to people in the trade. However, both the expected increase in palm oil production and concomitant fertilizer usage have to take full cognizance of worldwide environmental concerns on two major counts.

MATERIAL AND METHODS

In this study for examination of sulfur and magnesium on seed oil of *Phoenix dactylifera* 'Shahani' first, four levels of magnesium were used (0, 40, 80,120 mg Mg kg^{-1} soil) by $Mg(NO_3)_2$ and four levels of sulfur (0, 40, 80, 120 mg S kg^{-1} soil) by $(NH_4)_2SO_4$. These elements were added to the 40 cm of surface soil with 30 cm distance between trees. These treatments were added to the soil in the first half of February and irrigation was done by drip method. In the growing time some elements such as nitrogen, phosphorus and potassium by urea (150 kg ha^{-1}), triple super phosphate (100 kg ha^{-1}) and potassium sulfate (100 kg ha^{-1}) were added to the farm. In the end of September harvest took place and seeds were separated from fruits. Then the seeds were ground and made into a powder. Next 100 g of seed powder was added to 1000 g of water and put in the autoclave at 300°C for 60 min after which it was left to cool gradually. After that, separated oil and water were decanted and the weight of oil was measured in every treatment. Finally, the data were analyzed by software; the statistical design applied in this study was a Complete Randomized Design (CRD) with sixteen treatments and three replications. Means' comparisons were done by Duncan's test. For statistical analysis of data the computer software MSTATC was used.

RESULTS AND DISCUSSION

Result of this experiment are shown; increasing of magnesium levels had a positive significant effect on seed oil. So, with increasing magnesium levels the oil value was increased. The level of 120 mg magnesium had the most positive significant effect on seed oil compared to the control and other magnesium levels and this level increased seed oil by 15% related to the control. With an increase of the sulfur levels the oil value was increased. Levels of 80 and 120 mg sulfur had no significant effect with other, but they had a significant effect with other levels, especially the control. These levels increased the seed oil by 12% related to the control. The interaction of these elements with increasing levels of these elements, increased the seed oil. Most of the seed oil was related to the treatment with 120 mg magnesium and 80 mg sulfur. This treatment caused a positive significant increase compared to the control and other treatments.

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Tables

Table 1. Interaction of sulfur and magnesium on the seed oil of palm.

Mg / S	0	40	80	120
0	2.42e*	2.57d	2.69c	2.78bc
40	2.50d	2.61c	2.70bc	2.80b
80	2.71c	2.75bc	2.80b	2.86a
120	2.72c	2.77b	2.82ab	2.85a

* Numbers with the same letter are not significant at 1% level and increased the seed oil by 18% related to the control.

Potential of Commercial Cultivation and Expansion of Date Palm (*Phoenix dactylifera*) in Western Rajasthan, India

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Keywords: western Rajasthan, date palm, potential, Thar desert, expansion

Abstract

The vast geographical area in western Rajasthan state of Thar desert is climatically conducive for commercial cultivation of *Phoenix dactylifera*, commonly known as date palm. Availability of water for irrigation as well as thermal degree days in western Rajasthan are suitable for cultivation of this crop. Some of the cultivars among evaluated cultivars viz., 'Barhee', 'Halawi', 'Khadrawi', 'Khuneiji', 'Medjool', 'Khalas' and 'Migraf' are prominent and have indicated their suitability in this region. The spathe emergence begins in early February and tree ripened soft dates can be harvested by the end of June or early July. The maximum available thermal degree days are 185-190 in the extreme western district, Jaisalmer. Over the period of the last two decades a maximum production of soft dates to the tune of 150 kg/tree could be harvested in the cultivar 'Khadrawi'.

INTRODUCTION

Date palm is the oldest domesticated fruit crop from more than 5000 years ago in Mesopotamia, presently in Iraq. In the present situation, it has spread world wide through introduction and is prospected as major fruit crop for nutritional improvement and as an economic crop. The major commercial plantation of date palm has gone to Sindh in Pakistan during independence of India and Pakistan. However, the oldest commercial plantation exists in the Gujarata state in the coastal district Bhuj, where it occupies around 12,000 ha with major plantations of 'Barhee' (Mertia et al., 2006).

The research work on date palm was initiated in 1959 with introduction of female off-shoots of 31 cultivars in Abohar in Punjab and subsequently in a few places in Rajasthan after 1970. The heat summation unit required for ten prominent cultivars as mentioned in Table 1 (Chandra and Gupta, 1992) indicates their feasibility for commercial cultivation in the most western districts; Bikaner, Barmer, Jaisalmer, Jodhpur, Jalore, and part of Pali in the state of Rajasthan where additional 4.1 million ha is available for the crop.

The observation recorded on phenophases of prominent cultivars of date palm gives an idea about start of flowering, flower length, number of bunches/plant (Table 2) and about their production potential for commercial cultivation in desert district of Western Rajasthan, India.

CONDUCIVE SITUATION

Date palm cultivation has two basic requirements: heat summation unit above 3000 degree days and enough water for irrigation. Mertia and Faroda (1998) published elaborative articles on potential of commercial cultivation of date palm in cannal command areas in desert of India and it attracted the corporate sector, the government and farmers for this crop. In an effort in this direction, the Rajasthan Horticultural Development Society, Govt. of Rajasthan, has launched a massive project on development of two model farms and expansion of the crop area through involvement of farmers.

Water was a limiting factor in the Indian desert but now with inception of the

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Indira Gandhi Nahar project in the northern region which is estimated to irrigate 1.9 million ha it is the most prospected area for cultivation of date palm. The potential of groundwater through tubewell irrigation is another area and thirdly the command area of the Narmada canal in the adjoining district of Gujarat state may also offer a large area for this crop in the time ahead.

HOPES AND CAUTIONS

The area available and now further availability of tissue cultured date palm plants in Jodhpur seem two potential factors for promotion of this crop in farming communities in this desert. However, without the experience gained from the countries including Australia, Mexico, Namibia, Peru, South Africa, Spain and USA this crop could not attain commercial status. The Indian desert is a new area which may provide an environment free of any disease and pest as has been experienced in the last three decades but the other factors in view of future likely changes in climate, biological and economical may need to be addressed simultaneously for successful commercial cultivation. The available area is large but needs to be assessed in a holistic way for this crop for a future in the Indian desert (Mertia et al., 2010).

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Tables

Table 1. Heat summation unit required for promising date palm cultivars at Bikaner (from spathe initiation to full dang stage).

Cultivars	Heat summation units (base is 10°C)	
	Early doka	Full dang
Halawy	1951	3101
Medzool	2648	3650
Zahidi	2322	3479
Khalas	2357	3281
Barhee	2323	Not attained
Migraf	2244	3281
Zagloul	227	3631
Hayani	2460	3168
Shamran	2411	3567
Khadrawy	2213	3342

Table 2. Phenological observations on date palm during 2007-2008.

Cultivar	Spathe emergence	Spathe opening	Flower cluster length (cm)	No of bunches/ plant	No of straids/ bunch	Average length of straid (cm)	No of fruits/ straid
Dayani	06-03-08	19-03-08	95.3	5	31	29.7	15.0
Shamran	09-03-08	21-03-08	78.2	8	32	34.4	13.5
Khadrawy	12-03-08	19-03-08	72.6	9	30	32.8	10.9
Barhee	04-03-08	22-03-08	61.2	5	28	30.5	11.8
Migraf	28-02-08	11-03-08	70.2	4	30	27.2	10.2
Medzool	12-03-08	25-03-08	96.7	8	27	36.0	10.7
Saidy	10-03-08	21-03-08	72.2	9	33	23.4	6.9
Khalas	29-02-08	18-03-08	92.4	8	36	35.7	13.6
Umshok	28-02-08	16-03-08	58.8	9	21	25.4	10.8

The Role of Date Palm Tree in Improvement of the Environment

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Keywords: *Phoenix dactylifera* L., ecology, desertification, temperature, pollution

Abstract

Over the history date palm tree has played a major role in the life of human beings. It has been used as a source of food, building houses, and landscaping. It has been cultivated alongside rivers, springs, and wherever water is available. The date palm tree has played and is still playing a significant role in Muslims' life in general and Iraqi's in particular since it represents the food, shelter, garden, shade and orchard for them. The role of the tree has increased when growers realized that the tree is salt and drought tolerant, in addition to its impact in combating desertification. The tree can decrease the atmospheric temperature and the level of pollutants resulted from industrial activities. The symmetric shape of the date palm tree has added another dimension to its impact on future improvement of the environment. These aspects and others will be discussed through this paper.

INTRODUCTION

Global warming has changed the pace of our environment and is continuing to do so unless effective measures are taken. The earth used to be covered with water and forests just before the industrial revolution. Activities of humankind have resulted in huge devastation in his environment. The Middle East, particularly the Gulf States including Iraq are the witness of such changes, however they used to be the cradle of civilization or so called Mesopotamia and sometimes the Fertile Crescent. Date palm fruit undoubtedly was the staple food for Arabia over past times. The tree was a symbol of life since it offered food, shelter, house, bed, furniture and even trade for them over many centuries. The historic scripts of ancient Iraq mention how Otto, the God of sun, extracted sweat water from the ground in the land of Delmon (sailors trade with dates). People believed that Enki, the God of Aspo (underground water that the earth floats on) created the palm tree (Al Khalifa and Al Khalifa, 2004).

The date palm (*Phoenix dactylifera* L.) tree belongs to the *Palmaceae* family and has a long cylindrical, unbranched solid trunk, which ends with fronds. The tree has a big leaf area and hence large leaf area index reflecting a possible shade area underneath the tree approaching 15 m^2 or may be more depending on how vigorous the tree is. Its shade giving qualities are invaluable both for car parking and for pedestrians or informal recreational facilities. Accordingly, a decrease in ambient temperature beneath the tree reaches 10°C less that in the non-shaded area. The big feathered fronds allow for some irradiance to transmit to the soil surface allowing another shade loving crop to flourish. This would add additional merit by improving the farmer revenues. Such big leaf area defiantly absorbs considerable amounts of sunshine utilizing the photosynthetically active radiation, this in turn results in more photosynthesis and ultimately better fruiting, taking into consideration the abundance of CO_2 nowadays in our environment.

Revegetation and creeping towards the desert with date palm plantation will contribute in a tremendous fall in ambient temperature leading to huge savings in cooling costs attributed to the reduction in electricity consumption. Deforestation, shortage in irrigation water, bad agricultural practices, soil salinity, pollution, human activities and certainly the industrial revolution have caused devastation to our environment. Amin (2004) indicated the extent of desertification that has already widespread in many regions

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in Arabian countries including the Kingdom of Saudi Arabia. Date palm trees are however in challenge with a variety of adverse climatic conditions, when the tree is fully exploited. Soil salinity is another serious threat almost all over the world particularly in the land that used to be called the Fertile Crescent. The tree is classified as salt and dry tolerant (Clouston, 1978) and thus it is a possible candidate for salt affected soils that cover wide areas in dry and semi-dry regions of the tropics and sub-tropics (Ibrahim, 2010). It is worth to mention that there are some wider ecological advantages of using indigenous species wherever possible. These include the minimization of adverse ecological effects associated with the importation of pests and diseases from overseas and the local identity of such species (Fraser, 1983). Growing palms in such areas will lead to further improvement in climate taking into account the low transpiration rate of the tree due to the waxy layer that covers the fronds. Desserts are mostly bare lands exposed to high speed wind accompanied very often with dust upraise reaching urban cities. It seems that ecological systems are almost out of control unless decisive measures are taken. This seems clear even for people who lived for centuries near oasis that are scattered in the desert. They used to grow date palms surrounding the oasis (Xiaoling and Jiping, 2003). Date palm plantations have an additional advantage in improving soil physical and chemical properties due to the large amounts of organic materials deposit to the soil after the tree pruning. This would lead to a thriving niche for soil flora and fauna and therefore enriches the biological diversity in the proposed ecosystem and an important element in the ecological framework of a given region.

Landscaping is an aspect of concern where a harsh environment is dominant and does not sustain many plants of choice (Willens, 1977). A search for a tree or trees fulfill and meet such severe conditions is highly desirable, although gardeners realized this some time ago. Palms have been introduced as a key accentuating element in private gardens and parks design where shade and fruit are required alike, so that they have potential use in contemporary landscape design. Since palms are tall, they match tall buildings especially those with glass facades that people tend to construct nowadays so that they prevent glaring which may represent a serious risk for drivers.

In conclusion, the date palm tree is not fully utilized so far as much as the environmental issue is concerned. When it is thoroughly exploited it can do a lot; it combats desertification, reduces macro- and micro-climate temperatures, conserves soil from all types of erosion, encourages biological diversity, reduces pollutants particularly CO_2 and dust, works as windbreak, prevents glaring, in addition to its use for amenity purposes and so many other advantages.

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Effect of Bunch Removal and Fruit Thinning on Shriveling of Mature Dates in 'Ghar' Cultivar

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Keywords: date palm, bunch removal, fruit thinning, shriveling of dates

Abstract

Date palm (*Phoenix dactylifera*) is the most important crop in Saudi Arabia which accounts for nearly 15% of the global production of dates. Shriveling of dates as characterized by drying, wrinkling and underdevelopment of mature (tamar) dates causes substantial reduction in yield.

Trials were carried out during 2007 and 2008 to assess the effect of bunch removal (main treatment) and fruit thinning (sub treatment) on shriveling of matures dates in 'Ghar', a popular date palm cultivar in the Kingdom of Saudi Arabia. Data on the number of shriveled fruits per palm were subjected to analysis of variance using the two-factor factorial experimental design.

Results revealed significant differences among treatments. With regard to the main treatment of bunch removal the lowest number of shriveled dates (153) was recorded when 33.00% of the bunches were removed, while for the sub-treatment of fruit thinning the least shriveling (148) was recorded with 25% strand thinning. With regard to the overall treatment mean (interaction), 33.00% bunch removal coupled with 25% strand thinning recorded the lowest number of shriveled dates of 124 which was statistically at par with 25% bunch removal coupled with 25% strand cutting.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is the most important crop in Saudi Arabia and throughout the Middle East (Al-Hamdan, 2009). It provides a nutritious source of food, and fiber for shelter, clothing and furniture. With 25 million palms the Kingdom produces nearly a million tons of dates accounting for nearly 15% of the global production (http://www.en.wikipedia.org). Shriveling of dates as characterized by wrinkling, drying and underdevelopment of mature dates (tamar) leads to substantial loss in yield. However, there are no reports on the extent of shrivelling and also the causes of shriveling of dates in the kingdom of Saudi Arabia. Fruit load on the date palm has been attributed as one of the possible causes of shriveling of dates (Tahaer, 1983).

The Kingdom of Saudi Arabia has a rich diversity of date palm cultivars. Among these the cultivar 'Ghar' yields mature fruits early in the fruiting season during the month of June and is preferred by consumers in the two major date palm oasis of Al Qatif and Al Hassa in the Eastern Province of the Kingdom, where it constitutes 3-6% of the cultivated date palm cultivars (Fathi, 1979). Due to the heavy bearing tendency in 'Ghar' of up to19 bunches, each with an average of 3000 fruits , the cultivar is prone to breaking of bunches and also shrivelling of dates within healthy bunches resulting in substantial loss in yield (Tahaer, 1983).

This study was carried out in Al Qatif during 2007 and 2008 to assess the effect of bunch removal and fruit thinning on the extent of fruit shriveling in the popular date palm cultivar 'Ghar' of Saudi Arabia.

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MATERIALS AND METHODS

Trials were carried out at the research farm of the National Date Palm Research Centre (NDPRC), Al Qatif, Ministry of Agriculture, Kingdom of Saudi Arabia during 2007 and 2008, to ascertain the effect of bunch removal (main treatment) and fruit thinning (strand thinning) on shriveling of date palm fruit in the popular date palm cultivar 'Ghar' in the date palm oasis of Al Qatif in the Eastern Province of the Kingdom of Saudi Arabia.

During both 2007 and 2008, 12 palms of the cultivar 'Ghar' were selected for the trials. The experimental palms were vigorous, healthy, 20 years old and approximately 7 m tall cultivated at a spacing of 10×10 m (100 palms/ha). The date palms selected for the trial received standard agronomic practices (Ben Abdallah, 1990) with respect to irrigation, fertilizer application and plant protection routinely adopted at the farm of NDPRC at Al Qatif.

The trials were conducted through a two-factor factorial completely randomized block design, where bunch removal constituted the main treatment and thinning of date fruit strands from the remaining bunches was the sub treatment. Removal of bunches was adopted at four levels viz. 20, 25, 33 and 40% bunch removal while the three fruit thinning treatments were i) no thinning (control), ii) 25% strand removal from the bunch and iii) 25% cutting of the fruit strands from the bunch. In all there were 12 treatments each replicated thrice. Treatment codes for bunch removal was indicated on the trunk of each experimental palm, while the sub treatment of fruit thinning within the bunches retained was identified through a distinct colour code. Treatment details are presented in Table 1.

Bunch removal and fruit thinning were carried out eight weeks after pollination to coincide with the initiation of fruit setting. At fruit maturity (tamar stage) an estimate on the number of fruits per bunch was made by weighing each bunch and counting the number of fruits in 1 kg of mature dates. Further observations on the number of dry, wrinkled and underdeveloped fruits was recorded per bunch to estimate the number of shriveled fruits. Data on the number of shriveled dates per palm was subjected to ANOVA using the Web Based Agricultural Statistical Package WASP 1.0 consulted at http//www.icargoa.res.in. Results obtained are presented and discussed below.

RESULTS AND DISCUSSION

Results presented in Figures 1 to 5 indicate significant differences (p=0.05) among the treatment means of the main (bunch removal), sub (fruit thinning) as well as for the interaction means.

With regard to the main treatment of bunch removal it is seen from Figure 1 that 33% bunch removal recorded the least number of shriveled dates per palm (153) and was statistically at par with 25 (174) and 40% (160) bunch removal but significantly (p=0.05) different from 20% (206) bunch removal. In the sub treatment of fruit thinning the least number of shriveled fruits per palm (148) was recorded at 25% strand removal which was at par with 25% strand cutting (151), both of which were significantly different from the control treatment where no fruit thinning was done and where 221 shriveled fruits per palm were recorded.

With regard to the interaction effects it is seen from Figure 3 that the treatment Cb of 33% bunch removal coupled with 25% strand thinning resulted in the least number of shriveled fruits (124) and was statistically at par (p=0.05) with the treatment Bc of 25% bunch removal coupled with 25% strand cutting which recorded 144 shriveled fruits per palm. In all the interaction treatment means, the highest number of shriveled dates was recorded when only bunch removal was done without any fruit thinning. This signifies the importance of combining both bunch removal with fruit thinning for the efficient management of shriveling of dates in the cultivar 'Ghar'. This finding is in agreement with Nixon and Carpenter (1978) who reported that bunch removal and fruit thinning when done individually does not help to reduce shriveling of dates.

Tahaer (1983) reported increased shriveling of dates due to too many fruits per

bunch. Our findings signify the importance of fruit thinning in reducing shriveling of dates and are in agreement with the findings of Tahaer (1983) who suggested that too many fruits at high temperature during fruit maturity increases shriveling of dates.

CONCLUSION

The findings of this study reveal the importance of both bunch removal and fruit thinning in date palm at fruit setting for efficient management of shriveling of dates in the cultivar 'Ghar'. At least 25% bunch removal is essential to achieve significant reduction in shriveling of dates. Both, 25% strand removal and 25% strand cutting are efficient to reduce shrivelling in the fruit that reach maturity (tamar stage). However, when bunch removal was combined with fruit thinning, 25% bunch removal combined with 25% strand cutting was found to be adequate. These agro-techniques are therefore recommended to significantly reduce shrivelling of dates in the 'Ghar' cultivar of date palm.

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Tables

Table 1. Treatment details pertaining to bunch removal and fruit thinning.

Main treatment: bunch removal		Sub treatment: fruit thinning	
Code	Treatment	Code	Treatment
A	20% bunch removal	a	No fruit thinning
B	25% bunch removal	b	25% strand removal
C	33% bunch removal	c	25% strand cutting
D	40% bunch removal		

Figures

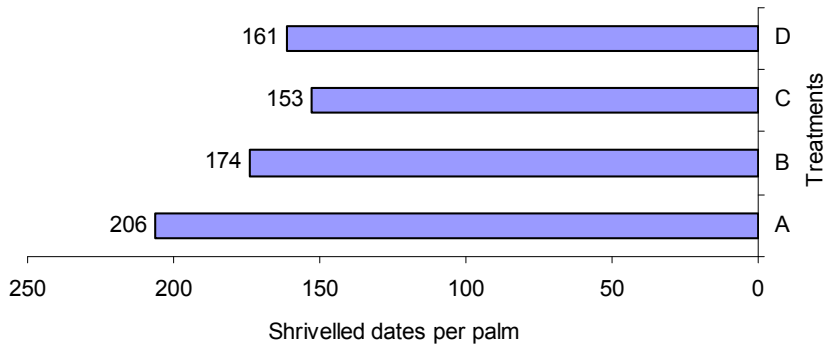

Fig. 1. Effect of bunch removal on shriveling of 'Ghar' dates [CD(p:0.05)=23.99].

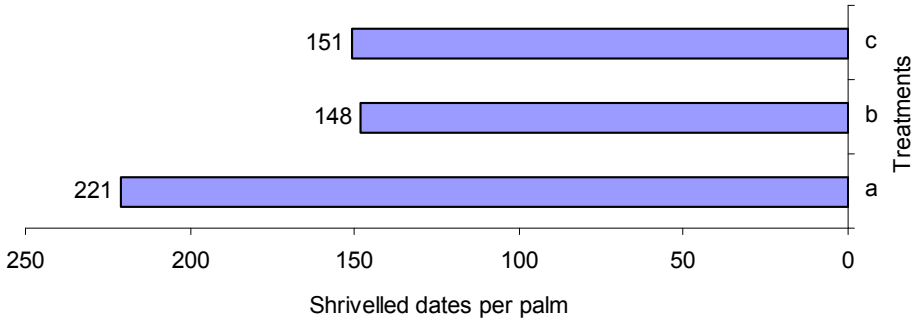

Fig. 2. Effect of fruit thinning on shriveling of 'Ghar' dates [CD(p:0.05)=20.77].

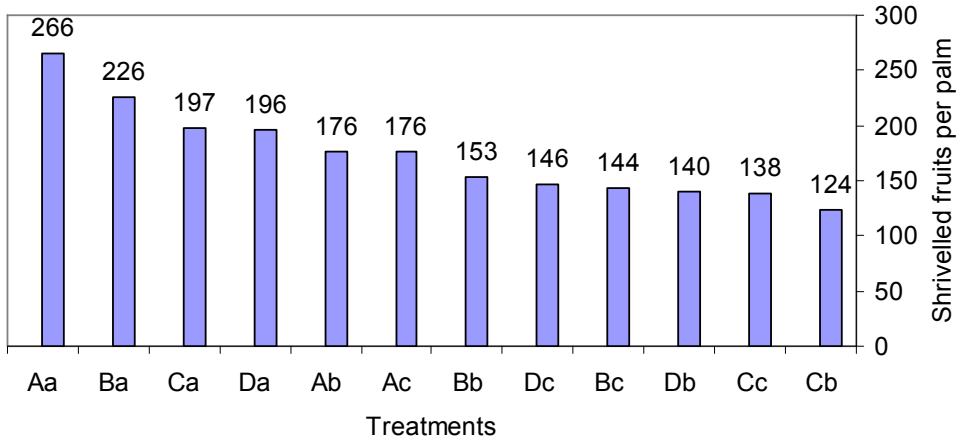

Fig. 3. Effect of bunch removal and fruit thinning on shriveling of 'Ghar' dates [CD(P:0.05)=41.55].

Fig. 4. Shriveling of ‘Ghar’ dates - normal fruits on the top and shrivelled fruits below.

Fig. 5. Shriveling of strands and dates of the cultivar ‘Ghar’.

Date Palm Genetic Diversity Conservation for Sustainable Production

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Keywords: plant genetic diversity, cryopreservation, cold storage, gene bank

Abstract

The rapid loss of genetic diversity has become a major concern worldwide for the genetic improvement of crops for sustainable agriculture especially under the climate change. Moreover, events like rapid human population growth, industrialization, deforestation, and natural calamities, are some of the major factors in this loss. The conservation, distribution, and utilization of natural and induced genetic diversity have become essential by the establishment of gene/germplasm bank both at the national and international levels. In vitro conservation techniques such as cryopreservation and low temperature storage are being routinely used for genetic resources conservation of a wide range of crops including seed propagated and vegetatively propagated crops. Cold-storage is done at 4-5°C and has a major disadvantage of frequent subcultures of in vitro cultures; may run into a risk of contamination. This method is routinely used for seed storage by national and international gene banks, maintained by CGIAR institutes, e.g., International Rice Research Institute (IRRI). Cryopreservation of genetic material is done in liquid nitrogen at -196°C on the long-term basis without going through frequent subcultures. In vitro cultures are suitable for cryo-storage, that includes somatic embryos, cell suspension, callus, and should be able to regenerate plants maintaining their genetic fidelity. In date palm, in vitro conservation work done is very limited. Nowadays, the date palm tissue culture system is well established via somatic embryogenesis and organogenesis, which is of course genotypic dependent on frequency of plant regeneration rate. Most commonly somatic embryogenic cultures have been used for cryo-storage of date palm genetic material.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is one of the most ancient cultivated plants since over 4000 years. The continuous selection for desirable traits by man has resulted in narrowing the genetic diversity, and has left hardly any scope for developing new cultivars with useful traits. Very few plant species have been so closely connected with the survival and well being of humans living in hot and arid environments. Also, the date palm tree has a great socio-economic importance and nutritional value in the Middle East and north Africa. In addition, date palm trees withstand adverse environmental changes such as drought, rains, flood, temperature fluctuations, and also help in preventing desertification. These drastic climatic changes could have adverse consequences by losing the genetic diversity. Date fruits are a very important source of human nutrition as well as an export item for date palm growing countries. Most importantly the plant contributes in the creation of a 'microclimate' within the fragile oasis ecosystem and allows development of agriculture in drought and saline conditions; multiple cropping system such as grapes, tomato and so on. The genetic diversity of date palm represents different local cultivars grown under varied climatic conditions. The most popular Sudanese date palm cultivars described as soft and dry types, showed detectable genetic variation in fruit morphology (Elshibi, 2009; Jain, 2011). Moreover no conventional methods e.g., seed storage were used to conserve genetic diversity due to high nature of heterozygosis for

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developing date palm breeding programs. With the development of in vitro culture techniques - organogenesis, somatic embryogenesis, embryo rescue-plant regeneration has already been accomplished, which is highly genotypic dependent; has made possible short- and long-term storage of in vitro cultures, large-scale multiplication of plants of elite genotypes (Jain, 2011).

CONSERVATION OF GENETIC DIVERSITY

Plant genetic diversity conservation has become an important issue among researchers worldwide for present and future agriculture. The rapid loss of genetic resources is mainly due to rapid industrialization, fire, deforestation, and environmental pollution. Moreover, lack of availability or non-existence of desirable genotypes hampers plant breeders for developing new cultivars. Seed source is commonly used to conserve plant genetic resources and stored at low temperature. It is quite cost effective, safer, and easy to handle. The second approach is in vitro conservation of in vitro cultures in cold storage and cryo-storage (Bekheet et al., 2001, 2005, 2007). The success of this approach is very much dependent on plant regeneration and the maintenance of genetic fidelity of the stored genotypes. Nowadays international germplasm exchange has become more cumbersome and sometimes it is rather difficult to obtain elite genotypes. Many countries do not share the germplasm because of patent and ownership problems, and have established national germplasm conservation or gene bank facilities. In India, the National Bureau of Plant Genetic Resources (NBGR) was established in Delhi to conserve local and imported genetic material for proper utilization in developing new cultivars. Countries like Costa Rica, South Korea, Thailand and others have established germplasm conservation facilities. Gene banks of CGIAR institutes worldwide are an important source of exchange of germplasm (Jain, 2011). In the European Union, there are several projects being carried out on germplasm identification, collection, conservation, and utilization of various crops, e.g., *Allium* spp., and have established websites of available genetic resources.

CONSERVATION OF INDUCED GENETIC DIVERSITY

The natural genetic diversity is gradually eroded and consequently loss of valuable genetic resources that hinders genetic improvement of crops including date palm. Moreover the rate of spontaneous mutations is extremely slow and makes the availability of wide genetic diversity harder for plant breeders. Therefore, induced mutations hasten the rate of genetic diversity in a short period and are readily available to plant breeders for developing new cultivars. Mutations are induced by physical (e.g., gamma radiation) and chemical mutagen (e.g., sodium azide) treatment in seed and vegetative propagated crops including the date palm (Jain, 2010a,b; Jain et al., 2010). The FAO/IAEA Mutant Variety Database (MVD) collects information on plant mutant varieties (cultivars) released officially or commercially worldwide (http://www.iaea.org). A mutant variety is a new plant variety that is bred through: 1. direct use of a mutant line that is developed through physical and chemical mutagenesis, or somaclonal variation; 2. indirect use of a mutant line/lines, which is/are used as a parental variety/varieties in cross breeding (cross between mutant lines or with a commercial cultivar/cultivars); 3. the use of a mutant gene allele (trait) Calrose 76 sd1 allele (semi-dwarf 1 trait) in rice; 4. use of wild species' genes translocated into plant genomes through irradiation facilitated translocations, e.g., genes of wheat wild relative species. In the FAO/IAEA mutant variety database, date palm mutants are yet to be available. However, mutation work on date palm has produced putative mutants showing tolerance to Bayoud disease (Jain, 2007).

TYPES OF GENE BANKS

Community Seed Bank

The community seed bank is quite common at the village level for the preservation of local cultivars and agriculture production in many developing countries. Farmers rely

on informal seed systems based on local growers' retention of seed from previous harvests, storage, treatment and exchange of this seeds within and between the communities. The informal seed sector is typically based on indigenous structures for information flow and exchange of seeds. Seed banks managed within this local seed system operate on a small scale at the community level with few resources. In date palm also the local high quality genetic material is conserved at the village or community level by preserving seeds. For more see http://www.bioversityinternational.org.

Seed Gene Banks

Seed banking is most widely used for the conservation of plant genetic resources. Initially the moisture content of seeds is lowered by drying them and they are stored at subzero temperatures in cold stores or deep freezers. Over 90% of global plant genetic material accession is stored by seed banking, e.g., all gene banks of the CGIAR institutes. The main problem with this technique is inability of seeds to withstand desiccation at lower temperatures and that may hamper seed germination rate or survival of seeds may seriously be affected and ultimately they die. For more see http://www.bioversityinternational.org.

Field Gene Banks

The genetic material is collected and planted in the field or orchard either in the same or different location. These gene banks have traditionally been used for perennial plants, including:

- species producing little or no seeds.
- species that are preferably stored as clonal material.
- species that have a long life cycle to generate breeding and/or planting material.
- Species producing recalcitrant seeds.

Crop species cocoa, rubber, coconut, coffee, sugarcane, banana, tuber crops, tropical and temperate fruits, vegetative propagated crops, such as wild onion and garlic, and forage grasses are most commonly conserved in field banks. Date palm would be ideal to conserve in a field gene bank. For more see http://www.bioversityinternational.org.

Bud Bank or Vegetative Banks

Seed banks are most widely used for genetic material conservation, and the role of the bud bank or vegetative bank has received little attention (Klimesona and Klimes, 2007). The term bud bank was coined by Harper (1977). The bud bank consists of all buds that can potentially be used for vegetative propagation/regeneration (for more details see Klimesona and Klimes, 2007). The advantage of bud banks is to exploit innate dormancy and induced dormancy, which is induced by drought or cold (Anderson et al., 2001). Another type of dormancy beneficial to bud banking is correlative inhibition which is represented by apical dominance. This mechanism prevents actively growing apical buds growth of axillary and adventitious buds situated below apical meristem. As a result buds remain available for vegetative regeneration until an injury breaks the apical dominance (Klimesona and Klimes, 2007).

In Vitro Storage Bank

In vitro storage, keeps plant tissues under strict sterile conditions, stored in petri dishes, glass tubes and vessels. By lowering the temperature above zero degree, plant tissue growth is drastically reduced and that minimizes frequent subcultures on fresh culture media. These banks are unsuitable for long-term storage of plant material. For species with so-called 'recalcitrant' seeds or species that are vegetatively propagated, such as roots tubers and aroids, different conservation techniques are used at low temperatures. For more see http://www.bioversityinternational.org.

Cryo-Storage Bank

In these gene banks, the living tissues are stored at ultra low temperature -196°C in liquid nitrogen for long-term storage as well as prevent tissue culture derived genetic

variation. Plant species with recalcitrant seeds or vegetatively propagated plant species are conserved. Nowadays a wide range of species can be routinely cryopreserved: banana (*Musa* spp.) (Panis et al., 2001), cassava (*Manihot esculenta*), bramble fruits (*Rubus*), pear (*Pyrus*), vegetables in the *Solanum* family, coffee (*Coffea arabica*), oil palm *(Elaeis guineensis*) and tea (*Camellia sinensis*).

INTERNATIONAL GERMPLASM BANKS

Several countries have established national germplasm banks for the conservation, utilization, and distribution of genetic material for research to develop new cultivars as well as to protect from their false claimers and bio-pirates, e.g., neem tree, basmati rice, and turmeric were claimed by some companies to have exclusive rights to use them, and the Indian Government challenged these companies, and finally won the case. High value genetic material is being protected by several governments for preventing false claims of greedy companies. Date palm germplasm conservation needs to be established for preserving high quality date genetic sources and establish a genetic data base of available date palm genetic material that would facilitate in developing new cultivars in date palm growing countries.

Gene banks at various CGIAR institutes have been established and conserve different types of crop depending on the location. Currently, CGIAR gene banks hold a total of 629,022 samples of crops and their wild relatives. There is no indication of a well established date palm gene bank under the CGIAR and in any date palm growing country. Most likely date palm growers maintain their own high quality genetic material without sharing with other growers, and also for improving the date palm quality. In the future, date palm could be used as a bio-energy crop for the production of bio-ethanol, and would give extra income to growers. The selection of appropriate date palm genetic material would be crucial for producing date fruits for food, feed, and bio-energy.

IN VITRO CULTURE OF DATE PALM

Traditionally, date palm is propagated both sexually through seeds and vegetatively by off shoots that produced from axillary buds situated on the base of the trunk during the juvenile phase of the date palm tree. It is quite slow for off shoots to develop and that hampers vegetative propagation of date palm trees. So far, there is no available technique to speed up in increasing the off shoot numbers as well as reduce the time in developing them. The use of off shoots preserves the true-to-type character of multiplied genotypes. Moreover, sexual propagation of date palm is unsuitable for commercial production/propagation of true-to-type value-added genotypes. It is due to the heterozygous nature of date palm seedlings and their dioecious nature (Jain, 2007). In addition, half of this progeny will be composed of male trees which are not distinguished before the flowering stage. The female plants will produce variable fruits and generally of inferior quality (Eke et al., 2005). Furthermore, the seed propagation method has another limitation that the growth and maturation of seedlings is extremely low, and therefore, date palm seedlings may begin to fruit after 8-10 years of plantation.

Organogenesis

The use of plant tissue culture techniques such as somatic embryogenesis and organogenesis is highly suitable for large-scale plant multiplication of vegetatively propagated crops. The success of this approach is very much genotype dependent. In vitro techniques have successfully been applied for plant propagation in a wide range of crops including date palm (Jain, 2007; Jain et al., 2011). Micropropagation via organogenesis is widely used for rapid clonal propagation of elite genetic material of date palm. The performance of micropropagated date palm seems to be better than conventionally grown plants in terms of yield, early flowering time, and quite uniform in fruit quality and physical properties. Aaouine (2003) reported plant regeneration from 30 genotypes of date palm via direct shoot organogenesis. The major concern with this approach is somaclonal variation that is dependent on various factors including genotype, explants,

plant growth regulators (Jain, 2001).

Somatic Embryogenesis

Somatic embryogenesis of date palm has been quite successful in plant regeneration (Fki et al., 2003; Al-Khayri, 2005). The most frequently used explants of date palm are apical shoot tips and lateral buds for successful plant regeneration (Jain, 2007). Smith and Aynsley (1995) studied field performance of tissue culture derived date palm clonally produced by somatic embryogenesis, and the results demonstrated that these plants started bearing fruits within 4 years from field planting of small plants with leaf length 100 cm and 1.5 cm diameter at the base. The main advantages of somatic embryogenesis are: ideal for cryopreservation, cost effective for large-scale propagation, and embryo production in a bioreactor.

IN VITRO CONSERVATION

The purpose of date palm genetic material conservation is to protect from deforestation, man-made environmental pollution, and natural calamities such as hurricanes, floods, drought, fire, etc. In Grenada, hurricane Ivan and Emily in 2004 and 2005 damaged 90% nutmeg and other spice trees, and resulted in loss of agriculture production, elite germplasm, and exports. The basic requirement of in vitro conservation and cryopreservation of genetic resources is the reliable plant regeneration from in vitro explants and large-scale disease-free plant multiplication. Failing in plant regeneration, this technique may be useless to store in vitro cultures. Most common in vitro cultures are being used such as shoot tips, callus, cell suspension, microspore and somatic embryos.

Conservation at Low Temperature

At low temperature, 0-5°C, growth of stored shoot cultures is slowed down and that reduces the number of subcultures on the fresh culture media without influencing the genetic stability of cultures. It allows to store cultures for several years as long as over 10 years depending on plant type. However, rooted shoots enhance storage time much longer, e.g., in strawberry shoot cultures that developed excellent roots could be stored for three years without change of culture medium under low light intensity and 4°C (S.M. Jain, pers. commun.). The growth rate can also be reduced by increasing the sucrose concentration or addition of mannitol or sorbitol in the culture medium. Bekheet et al. (2001) were successful in the conservation of in vitro tissues including shoot buds and callus cultures of date palm ‘Zaghloul’ by slow growth method for 12 months at 5°C in the darkness. In vitro conservation has many advantages: disease-free planting material, high plant multiplication rate, all year round plant supply to the growers, potential of producing low cost planting material, and maintain the genetic fidelity verified with molecular markers. The major disadvantages of in vitro conservation are: loss of genetic material by contamination due to bacteria, fungi, virus and mites; subcultures on the fresh culture medium; labour intensive; destruction of stored genetic material due to fire or earth quake; and power supply interruptions. Therefore, utmost precaution should be taken to use healthy plant tissues for storage, and also test for virus-free material especially for example in cassava, strawberry and so on before initiating in vitro cultures for storage.

Cryo-Storage or Cryopreservation

Cryo-storage or cryopreservation is widely used for long-term storage of in vitro cultures of genetic material under ultra low temperatures, usually at -196°C in liquid nitrogen (Mycock et al., 1995; Bekheet et al., 2007; Subaith et al., 2007). This method preserves contamination-free material and prevents somaclonal variation. Since date palm in vitro culture has been worked out for plant regeneration, several groups have been engaged in cryo-storage of date palm tissues such as shoot tips, nodular cultures, callus, and somatic embryogenic cultures (Bekheet et al., 2007). Cryoprotectant treatment is given before plunging the tissue in the liquid nitrogen to prevent ice crystal formation in

the tissue in order to avoid any damage to the tissue that may adversely affect plant regeneration upon thawing of cryo-stored material. The common cryoprotects are polyehthylglycol (PEG), glucose, and dimethylsulfoxide (DMSO). In date palm, somatic embryo growth remains normal when treated with a cryo-protectant mixture of glycerol and sucrose. The growth rate or germination rate of somatic embryos should remain normal after the cryopreservation and that would reflect any adverse impact of various treatments during the following the protocol. Cryopreservation has applications for the elimination of viruses, which is also termed as cryo-therapy. Several viruses have been eliminated from various plants such as cucumber mosaic virus and banana streak virus from banana (Helliot et al., 2002), grape virus A (GVA) in vitro-grown shoot tips of *Vitis vinifera* L. (Wang et al., 2003), potato leafroll virus (PLRV) and potato virus Y (PVY) from potato shoot tips (Wang et al., 2006). The cryopreservation method allows only the survival of small areas of cells located in the meristematic dome and at the base of the primordial (Helliot et al., 2002). Therefore, cryo-therapy would be an alternative efficient procedure to eliminate viruses to produce virus-free plant material and simultaneously long-term storage of genetic material.

CONCLUSION

Date palm provides a nutrition source to the consumers in date palm growing countries. It is capable to withstand environmental stresses and provides a micro-climate in the desert for multiple cropping. Date palm has a great potential to provide renewable energy and could be used as a bio-energy crop for bio-ethanol production. There is a great need to prevent the loss of genetic diversity as the economic growth rapidly encroaches arable land. Moreover, the genetic base of date palm has narrowed down due to continuous selection, and that reduces further scope of genetic improvement of date palm. Thereby, conservation, distribution and proper utilization of date palm genetic diversity/resources have become necessary for sustainable crop production by developing new cultivars. Gene/germplasm banks should be established in order to facilitate easy accesses of genetic material to plant breeders. Also, a website should be developed for a date palm gene bank to provide all detailed information on each stored genetic material. Mostly date palm is vegetatively propagated, in vitro conservation of genetic material is an ideal approach for both short- and long-term storage. Moreover, date palm tissue culture is well established, which is essential while using the in vitro conservation method. Cryopreservation is well suited for long-term storage of somatic embryogenic cells and a reliable approach to control different type of viruses to produce virus-free material, and prevent somaclonal variation. In vitro conservation and cryopreservation of date palm is feasible since date palm tissue culture is well established for plant regeneration via somatic embryogenesis and organogenesis.

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Analysis of Sugars and Organic Acids Contents of Date Palm (*Phoenix dactylifera* L.) 'Barhee' during Fruit Development

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Abstract

Sugar and organic acid composition of date palm fruit (*Phoenix dactylifera* L.) cultivar 'Barhee' were measured using high performance liquid chromatography (HPLC) during fruit growth and development. According to the results, malic acid was detected as the main organic acid in the first growth stages while succinic acid and acetic acid were main acids detected in the Khalal and Tamar stages respectively. A reduction of total acid was observed until 100 days after full bloom (DAFB), and then dramatically increased during last growth stages (120, 140 and 160 DAFB). In these stages, acetic acid was the main acid increased and reached its maximum. As for the sugars, fructose and glucose were identified as the dominant sugars, and their levels varied remarkably during fruit development. The concentration of sugars was increased continually during fruit development reached its maximum level by 160 DAFB. Sucrose content was practically negligible.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) belongs to the *Arecaceae* family and its fruit is one of the oldest known edible fruits grown in many subtropical countries especially in the Persian Gulf region. The date fruit is consumed fresh and also used in the preparation of syrup, jams, table jellies, paste and dried date (Barreveld, 1993). The nature and concentration of sugars and organic acids in fruits have been of interest because of their important influence on the organoleptic properties and their important roles in maintaining fruit quality and determining nutritive value. Food analysts and plant physiologists have been interested in changes of the different chemical components occurring during fruit development and maturation (Wrolstad, 1981; Ashoor and Knox, 1982).

The physicochemical changes of different horticultural crops have been reported including sugars and organic acids. The relationships between these compounds and fruit ripening have been investigated in different fruits such as medlar (Glew et al., 2003), peaches (Wu et al., 2005), nectarines (Jacop et al., 2005), mango (Medlicott and Thompson, 2006), European and Asian pear (Arzani et al., 2009).

Date is a berry kind fruit, distinguished from most other fruits with a number of distinct growth phases including 'hababook', 'kimri', 'khalal', 'rutab' and 'tamar'. 'Barhee' is one of the most important and commercial date cultivars in the world. Its fruit can be consumed in the three distinct commercial maturation stages especially at khalal stage, when most other date cultivars are inedible (Glasner et al., 2002). In addition, the knowledge of the qualitative and quantitative chemical composition of date fruit is of prime importance to the dates consumers, in particular the packer, processor or traders. Most studies have been undertaken on date fruit compositional analysis focused on sugars, and little data are available in the literature concerning the organic acid composition of date fruits (Mrabet et al., 2008; Myhara et al., 1999; Ahmed et al., 1995). The objective of this work was to quantify the sugar and organic acid concentrations of date fruit 'Barhee' during development and maturation from 40 DAFB until the time of fruit drop at tamar stage under Ahvaz, Iran environmental conditions.

MATERIALS AND METHODS

Plant Material

Fruits of date (*Phoenix dactylifera* L. 'Barhee') were randomly collected in spring to summer 2006 from mature fifteen-year-old trees grown at the commercial orchard, Ahvaz (31°21'N; 48°40'E), Iran. The time of full bloom was recorded on 8 May 2006 followed by the seven fruit growth and development stages included first kimri, mid kimri, late kimri, first khalal, late khalal, rutab and tamar stages at 40, 60, 80, 100, 120, 140 and 160 DAFB respectively. The collected fruit samples were then transferred to the laboratory, then the fruit flesh was cut in the small pieces, immerged in liquid nitrogen and immediately held in the freezer (-80°C) until analyzed.

Chemicals

Phosphoric acid, acetonitrile and water (HPLC grade) were purchased from Merk (Germany) and Caledon (Canada) respectively. Sugars and organic acid standards were purchased from Supelco (USA). All other chemicals used were analytical reagent grade.

Extraction and HPLC Evolution

For sugar analysis, approximately 10 g of frozen sample was lyophilized using a freeze dryer (Chaist, Alpha 2.4), and then grinded to a smooth paste. 1 g dry weight of the ground sample was weighted and transferred to a falcon tube and then 5 ml of HPLC grade water added. The reaction mixture was placed in an ultrasonic bath and sonicated for 30 min at 40°C, then centrifuged at 8000 rpm for 15 min at 10°C. The supernatant was filtered through membrane filter (0.45 µM), transferred into a vial and used for sugar analyses. A 20 µl sample of filtrate was injected into the HPLC (Waters, USA), equipped with a 2414 RI detector, connected with Empower software and C-18 column (300×7.8 mm). The mobile phase consisted of acetonitrile-water (70:30, v/v), at a flow rate of 1 ml min^{-1} at 30°C (Arzani, 1994).

The procedure of Wu et al. (2005) was slightly modified and used for organic acid extraction and analysis. Frozen fruit samples were ground to a fine powder with the aid of mortar and pestle. 5 g of the obtained powder was mixed with 20 ml of ultra-pure water. The mixture was placed in an ultrasonic bath for 10 min at 5°C and then centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was recovered and immediately filtered through a Sep-Pak C18 cartridge (Millipore, Milford, USA) to eliminate any interfering polar residues and through a 0.45 mm filter to eliminate large particles (AOAC, 1995). The analysis was carried out by injecting 20 µl of extracted samples into the Waters HPLC system with an Empower software, a pump (Waters 600), using a Prontosil 120-3-C18 AQ column (250×4.6 mm) from Knauver (Germany). Column effluents were monitored by a UV-VIS detector (Waters 2487) set at 206 nm. The mobile phase used for the determination of organic acids was a solution of 50 mM phosphoric acid and the flow rate was 0.7 ml min^{-1}.

Data Analysis

For the stock solution of the organic acid standards, ascorbic, malic, oxalic, shikimic, succinic, glutamic, formic, lactic, tartaric, maleic, acetic, citric and fumaric acids were dissolved in methanol at a concentration of 1 mg ml^{-1} and the sugar standards, glucose, fructose and sucrose were dissolved in water at a concentration of 10 mg ml^{-1}. Sample concentrations were established by the absorbance recorded in the chromatograms relative to external standards and expressed in g/100 g FW (fresh flesh weight) for sugars and meq/100 g FW for organic acids. For each developmental stage, four replications were conducted and two injections were performed for each sample. Replication data of injections were averaged and extrapolated for different developmental stages during the monitored growth period. For selected series, a one-factor analysis of variance (ANOVA) was calculated using MSTATC statistical package (MSTATC, Michigan State University, East Lansing, MI) software. Means comparisons were made

using Duncan's multiple range test (DMRT); differences were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Like other fruits, the ratio of sugars to organic acids is considered as an index of quality and plays an important role for the fruit flavor and its marketability (Cordenunsi et al., 2002). In this study the sugar and organic acid composition of date fruit 'Barhee' were determined during fruit development using HPLC. A representative chromatogram of sugars and organic acids in date fruit is shown in Figure 1. Based on the analysis of variance (Table 1), the concentrations of studied sugars and organic acids among the growth stages were found statistically significant ($p<0.05$).

Sugar Analysis

The results were showed that all sugars in date 'Barhee' consist of a mixture of sucrose ($C_{12}H_{22}O_{11}$), glucose ($C_6H_{12}O_6$) and fructose ($C_6H_{12}O_6$). Fructose and glucose were identified as the principal sugars and sucrose was detected in negligible concentrations in all studied stages. It can be due to the fact that it may be converted to invert sugars such as glucose and fructose (Kafkas et al., 2006). Individual sugar contents correlated well with the sweetness characteristics of the fruit and the concentration of glucose and fructose increased continuously through development reaching their maximum at harvest time (31.01 and 32.23 g/100 g fresh weight respectively). These concentrations had markedly changed compared to the primary growth stages (Table 2). Also total sugar increased from 8.40 to 63.24 g/100 g fresh weight during fruit development.

As shown in Table 2, the glucose concentration was a little more until 100 DAFB, and then fructose concentration was found to be slightly dominant compared with glucose in the rest of the growth stages. The ratio of fructose/glucose changed from 0.83 to 1.03 during developmental stages when the concentrations of glucose and fructose varied between 3.89-32.23 and 4.51-31.01 g/100 g respectively. Sugar accumulation, especially the concentration of a high level of fructose and glucose is a very important physiological process that determines the dessert fruit quality (Kafkas et al., 2006). Due to the fact that fructose is about two-fold sweeter than glucose, the fruit sweetness contributes well with the fructose concentration especially when the fructose/glucose ratio increased to 1.03 in the three last developmental stages. It is noticeable in Table 2 that the rapid accumulation of glucose and fructose started from the khalal stage onwards and this is in agreement with the statements of Myhara et al. (1999). The rapid increase in the glucose and fructose concentration in last growth stages can be in relation of invertase activity that inverts sucrose into monosaccharide sugars (Hasegawa and Smolensky, 1970). The concentrations of mean fructose and glucose and total sugars among the different growth stages were found statistically significant ($p<0.05$). The number of sugars identified and their quantities in this study were in agreement with the results of Al-Farsi et al. (2005) for some Omani date cultivars. Also our results can be in corroboration with the results of Ahmed et al. (1995). They reported that the total sugar content in 12 United Arab Emirates date cultivars varied from 44.3 to 64.1 g/100 g and fructose/glucose ratio was about 1 for dates in the tamar stage. There have been some other reports attributed to increasing levels of sugars at advanced stages of date fruit maturity (Ahmed et al., 1995; Jaddou and Al-Hakim, 1980).

Organic Acid Analysis

The organic acids that give fruits their characteristic tartness vary in combination and in concentrations among different species and cultivars. The HPLC/UV analysis of organic acids showed that acetic, succinic, malic and tartaric acids are considered to be major organic acids in date fruit 'Barhee'. The concentrations of these individual organic acids in date fruit during different growth stages are given in Table 3. As shown in this table, malic acid, succinic acid and acetic acid were determined to be prominent organic

acids in the kimri, khalal and rutab stages respectively. Citric acid, shikimic acid and ascorbic acid were detected in negligible concentrations in all developmental stages. The malic acid concentration exhibited fluctuating patterns of seasonal changes and its amounts dropped from 13.11 (first kimri) to 2.03 (first khalal), then increased to 8.87 meq/100 g FW at the tamar stage. The succinic acid was the main organic acid in the khalal stage (11.91 meq/100 g FW) then decreased to 10.11 and 8.12 meq/100 g FW in the rutab and tamar stages respectively.

Succinic acid was the other major organic acid found in the last three studied stages and it reached a maximum concentration of 11.91 meq/100 g FW in the late khalal stage. The concentration of acetic acid increased rapidly from the first khalal stage with time until a maximum of 45.61 meq/100 g FW in the tamar stage. The concentrations of maleic and fumaric acids were very small in all developmental stages and the maximum concentrations of these acids were detected in the tamar stage (Table 3). The acidic property of organic acids is due to the carboxyl group (COOH) in a free state. These acids are an important source of fruit flavor mostly in combination with sugars. Most of the organic acids are probably present in the vacuole of the cell and they are respiratory substrates in the fruit (Ladaniya, 2007).

As shown in Figure 2, generally individual and total sugar concentrations showed positive correlations with acetic acid concentrations. It can be concluded that due to high sugar concentration and water content of 'Barhee' date fruit in the last developmental stages, acetic acid bacteria utilize glucose to produce gluconic acid and ethanol to produce acetic acid (Dufresne and Farnworth, 2000). This evolution in organic acids especially acetic acid was responsible for noticeable changes of sourness of 'Barhee' date fruits when they were left in the ambient air very soon. The acetate quantity depends mainly on the concentration of carbohydrates and the source of nitrogen as well as the pH (Fleet, 1994). The acetic acid content increases significantly during alcoholic fermentation if the sugar concentration is high (Erasmus et al., 2003).

The organic acids found for 'Barhee' in this study were partially in agreement with acids reported by Al-Farsi et al. (2005) for three Omani date cultivars at the tamar stage. They identified that six organic acids, among which malic acid was the predominant, followed by lesser amounts of succinic acid, isobutyric acid, citric acid, oxalic acid, and formic acid. Observed differences may be due to cultivation condition, soil type or method used for analysis. The acidity increase in the last developmental stages of date fruit was also stated by Golshan Tafti and Fooladi (2005) for 'Mozafati' date cultivar.

CONCLUSIONS

This study yielded information about the sugar and organic acid contents of date palm fruit 'Barhee' during different growth stages that might play a significant role in its flavor and shelf life. The data presented that sugars in 'Barhee' date are the most important fruit components and among the detected sugars, fructose and glucose were found to be the most abundant in all stages, the ratio of fructose/glucose concentration was found about 1 in the last stages. As for the detected acids, malic, succinic and acetic acids were found as the major ones and to our knowledge this is the first time that acetic acid has been determined as a main organic acid in a cultivar of date fruit. Although there is little information about the process of fruit fermentation in date fruit during the last growth stages, especially for 'Barhee', due to the high sugar concentration besides the water in date fruit, it can be surmised that the marked increase in acidity can be caused mainly by the yeast cells synthesizing acetic acid. During alcoholic fermentation when the sugars concentration is high, if yeast is subjected to osmotic stress, the reduction of acetaldehyde to ethanol is frequently observed to slow down, as a result of which more acetic acid is synthesized (Erasmus et al., 2003).

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Tables

Table 1. The analysis of variance for organic acids and sugars.

S.O.V.	D.F.	Mean square								
		Organic acids							Sugars	
		Oxalic acid	Tartaric acid	Maleic acid	Acetic acid	Malic acid	Succinic acid	Fumaric acid	Fructose	Glucose
Block	3	0.019^{ns}	0.460^{ns}	0.000^{ns}	1.142^{ns}	1.128^{ns}	2.080^{ns}	0.000^{ns}	0.915^{ns}	0.444^{ns}
Treatment	6	3.989^{*}	5.728^{*}	0.008^{*}	1166.420^{*}	63.677^{*}	97.364^{*}	0.000^{ns}	602.524^{*}	507.382^{*}
Error	18	0.020	0.222	0.000	5.616	0.906	1.763	0.000	0.443	0.357
Total	27									

Table 2. Sugar content (g/100 g fresh weight) of date fruit at various stages of development. Results are expressed as the means±SD of four replications.

Sugar	First kimri (40 DAF)	Mid kimri (60 DAF)	Late kimri (80 DAF)	First khalal (100 DAF)	Late khalal (120 DAF)	Rutab (140 DAF)	Tamar (160 DAF)
Glucose	4.51±0.13	4.33±0.59	5.64±0.22	7.72±0.24	13.94±0.91	27.43±0.33	31.01±1.08
Fructose	3.89±0.05	3.54±0.36	3.90±0.11	6.86±1.07	14.41±0.92	28.49±0.38	32.23±1.14
Fruc./Gluc.	0.83	0.81	0.69	0.88	1.03	1.03	1.03
Total	8.40±0.17	7.87±0.94	9.54±0.33	14.59±1.30	28.35±1.82	55.92±0.71	63.24±2.22

Table 3. Organic acid content (meq/100 g fresh weight) of date fruit at various stages of development. Results are expressed as the means±SD of four replications.

Organic acid	First kimri (40 DAF)	Mid kimri (60 DAF)	Late kimri (80 DAF)	First khalal (100 DAF)	Late khalal (120 DAF)	Rutab (140 DAF)	Tamar (160 DAF)
Oxalic acid	0.27±0.04	0.28±0.02	0.29±0.02	0.27±0.04	0.32±0.02	0.63±0.06	1.64±0.05
Tartaric acid	1.69±0.22	1.38±0.09	1.51±0.17	1.50±0.32	2.17±0.13	2.84±0.41	4.72±1.18
Maliec acid	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.04±0.00	0.12±0.03
Acetic acid	5.09±0.55	4.95±0.32	3.89±0.51	3.99±1.13	8.12±0.53	32.42±4.00	45.61±3.35
Malic acid	13.12±1.15	6.35±0.58	3.28±0.41	2.03±0.58	2.33±0.20	6.03±0.73	8.87±1.95
Succinic acid	0.52±0.11	0.88±0.10	1.29±0.17	1.53±0.22	11.90±0.99	10.10±2.47	8.10±2.33
Fumaric acid	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00

Figures

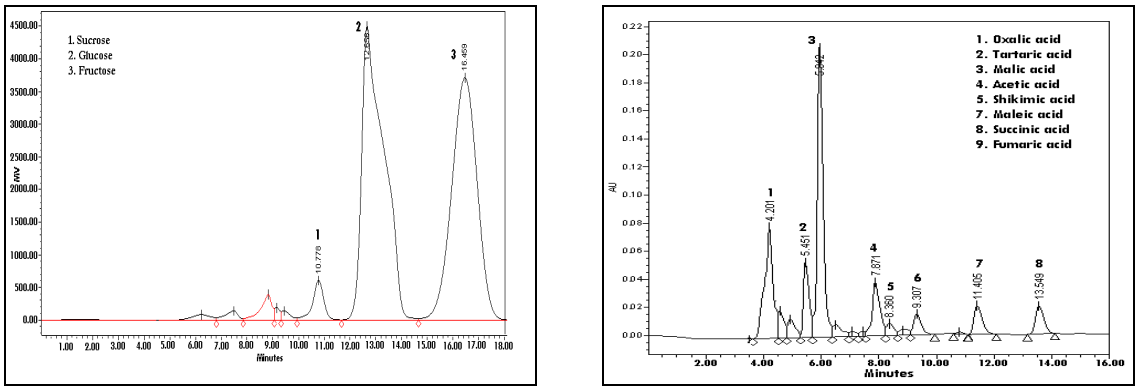

Fig. 1. HPLC profile of sugars (left) and organic acids (right) in 'Barhee' date fruit.

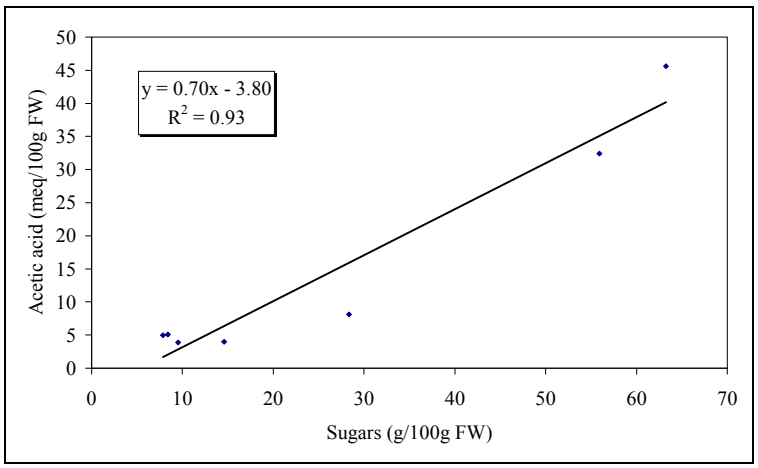

Fig. 2. Correlation between sugars and acetic acid changes during fruit developmental stages

Report of the Survey Studied on Somaclonal Variations in In Vitro Propagated Date Palm Plants

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Keywords: date palm, tissue culture, somaclonal variation

Abstract

In vitro production using either the process of somatic embryogenesis or organogenesis has been established in recent years as a routine procedure in several commercial laboratories to produce large numbers of date palm plants at a competitive cost. The date palm micro-propagation process, like other large-scale commercial plant production processes, carries a number of risks. Off type, i.e., non true to type and genetically not identical to the mother plant, may be among the resulting plants. This report was a survey studied in a date palm orchard containing 2000 plants of two cultivars ('Piarom' and 'Mejdhool') of five years old of in vitro propagated date palm plants. Various somaclonal variations appeared. Different growth rate and differences in plant shape appeared at a high level in the two cultivars. Dwarfism, morphological abnormality, chlorosis in one side of the leaf, growth point bending, leaves rotating, leaflet chlorophyll losses (albinism or variegation), abnormality of growth point, leaf malformation, death of terminal growth point (hypoxanthic palm), high vigorous plants and plants with poor establishment were observed in the two cultivars.

INTRODUCTION

Date palm (*Phoenix dactylifera*) can be propagated by seed, offshoot and via in vitro culture. The traditional method of date palm propagation is by offshoots. However, this method presents many disadvantages. Offshoots are produced in a limited number, the survival rate of offshoots is low, with high changes of spreading date palm diseases and pests, and the technique is difficult and laborious (Kunert et al., 2003). The most recent method for date palm propagation is tissue culture which presents many advantages such as the propagation of healthy selected female cultivars, producing males with superior pollen, large scale economic affordability when large production is needed (Zaid and Al-kaabi, 2003). In vitro propagation should normally produce true-to-type plants. However, the appearance of off-type plants has been observed. This process is referred to as somaclonal variation. It occurs in plants as a result of tissue culture conditions, and produces plants different from the original plant (Kaeppler et al., 2000). The existence of genetic variability in plant tissue culture has been reported in many plant species including date palm. The abnormalities found include changes in morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, leaf whitening, production of bastard offshoots, delayed flowering time, fertilization failure, formation of seedless fruits and higher susceptibility to diseases (Al-Mazroui et al., 2007; Cullis, 1999; McCubin et al., 2000; Zaid and Al-Kaabi, 2001, 2003). The in vitro production of date palm via somatic embryogenesis requires the application of relatively high concentrations of auxin-type plant growth regulators, such as 2,4-D or NAA, for process initiation (Bhaskaran and Smith, 1995; McCubin et al., 2000). However, these auxins are known to be associated with genetic instability in plants, and have become a known cause of genetic variability in date palm (Al-Mazroui et al., 2007; Cullis, 1999). Furthermore, variation in DNA methylation may be an important factor in initiating genetic variation (Cullis, 1992; Cullis and Kunert, 2000; Sala et al., 2000). Zaid and Al-Kaabi (2003) found

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that there were several morphological abnormalities in tissue cultured derived date palms, including abnormal leaves and inflorescences, dwarfing, leaf blanching, deformed offshoots, delayed flowering time, pollination failure and abnormal fruiting. Abnormal leaf shape and leaf bending were reported by Al-Ghamadi (1993) in different date cultivars produced using somatic embryogenesis.

MATERIAL AND METHODS

A survey aimed at assessing the morphological abnormalities was performed during 2007-2008, and covered 2000 date palm plants (6 years old) 'Medjool' and 'Piaroom' in Jahroom at I.R. Iran. The survey aimed to record the main abnormalities observed in cultivated date palms obtained via tissue culture and compared with date palms obtained from offshoots.

RESULTS AND DISCUSSION

Date Palm Morphology and Structure

Morphological abnormalities were found in cultivated two cultivars. These included: abnormal leaf phyllotaxy; thin stem with weak, juvenile leaves; absence of an onion like base and plants with rolled leaves which was observed in up to 40% of plants. The occurrence of these abnormalities at rate of up to 63% in a date palm nursery containing 2000 hardened in vitro 'Medjool' plants was observed in UAE (Al-Kaabi et al., 2007). By contrast, a recently established date palm orchard in Namibia contained only 5% 'Medjool' plants with abnormal characteristics (Zaid and Arias, 1999). Al-Mazroui et al. (2007) reported that the occurrence of abnormal morphology and structure in 5 cultivars of tissue cultured derived date palms in UAE was only very low and did not exceed 0.5%.

Dwarfism

Dwarf date palm plants are less than 1 m after 5 to 6 years in the field in comparison to a normal plant of the same age with an average height of 4 m in the two cultivars. The highest level of dwarfism (3%) was in 'Piaroom' and the lowest (0.5%) was in 'Medjool'. Zaid and Al-Kaabi (2001) found that certain date palm cultivars such as 'Sukari', 'Barhee', 'Sultana' and 'Oum Dahn' exhibited abnormal dwarfing. Djerbi, (2001) reported that dwarfism frequency was affected by cultivar type. The causes of dwarfism in date palm are not known. A dwarf phenotype is also associated with black scorch disease. Black scorch, also called Medjnoon or Fool's disease is caused by the pathogen *Ceratocystis paradoxa* (Amira et al., 2000). Black scorch-affected date palm trees can recover by chemical treatments but date palm plants derived from tissue culture are apparently more susceptible to this disease than offshoots and immediately after attack development of their meristems is restricted (Al-kaabi et al., 2007).

Excessive Vegetative Growth

An abnormality found in the date orchard that was surveyed was an excessive degree of vegetative growth. These plants were found to have broader leaves, compact growth and a different spine structure. Only 0.5% of 'Piaroom' was observed with this abnormal characteristic. MacCubin et al. (2001) found a much higher ratio of 1.4% in a survey carried out in South Africa on 'Medjool' plants that had been produced by three separate tissue culture laboratories using somatic embryogenesis.

Leaf Blanching / Albinism

Blanching of leaves was observed only in 'Medjool' and was due to partial or total loss of chlorophyll. Usually, 2 to 4 leaves were affected per tree. However, this abnormality was found to be rare in orchard (affecting 15 out of 200 plants surveyed) and is therefore of no great economic significance.

Deformed Offshoots

It is well known that date palm plants derived from tissue culture have a better growth habit and produce more uniform date palm orchards than those from offshoots (Smith and Ansely, 1995). They also produce more primary and secondary offshoots. However, this fast growing habit and the abundance of offshoots accompanied by the appearance of abnormal offshoots and twisted inflorescences. The frequency of these abnormal offshoots was approximately 25% of plants. This deformed condition can be caused by an infestation with the date palm bud mite (*Makiella phoenicis* K.), or may be due to reduction in growth caused by an inequilibrium of endogenous growth regulators accumulated during in vitro propagation (Hajian, 2007).

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Figures

Fig. 1. Dead of apical meristem.

Fig. 2. Multiple head.

Fig. 3. Differences in growth rate.

Fig. 4. Albinism.

Fig. 5. Excessive vegetative growth.

Fig. 6. Rolled leaves.

Fig. 7. Leaf scorch.

Fig. 8. Abnormal leaves.

Fig. 9. Abnormal growth.

Fig. 10. Dwarf ‘Medjool’ (5 years old).

Fig. 11. Unknown abnormality.

Fig. 12. Deformed offshoots.

Induction and Evaluation of Variability in Embryogenic Cultures of Date Palm (*Phoenix dactylifera* L.)

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Keywords: date palm, tissue culture, Sudan cultivars

Abstract

The study aimed to survey and to evaluate the existing date palm cultivars grown in Sudan. Also, cultural requirements were studied. Seventeen local cultivars were surveyed in northern Sudan and tissue cultures were initiated using axillary buds as explants. Cultural requirements were worked out for three cultivars ('Kulma', 'Mishrig-Wad-laggai' and 'Kursha'). The culture medium used was Murashige and Skoog medium supplemented with various combinations of auxins (IBA and NAA) and cytokinins (BAP and kinetin). A high auxin concentration (100 ml/L IBA) and 3 ml/L BAP yielded a white nodular callus from the explants of the 3 cultivars. The different calluses were anatomically examined for their cell composition. Cultured characteristics, callus morphology and cytology were recorded.

INTRODUCTION

Date Palm (*Phoenix dactylifera* L.), an important fruit crop in the *Arecaceae*, is traditionally propagated by offshoots, a method ineffective for the production of the required number of rooted, detachable off-shoots, of desired cultivars at specific times. Clonal propagation of commercially desired or locally selected date palm cultivars has been an objective which date palm growers and researchers have tried to achieve over the years.

Multiplication of true-to-type offshoots of date palms generation after generation without any change in genetic material, indicate that the extent of variability in the existing gene pool of date palm cultivars is very narrow, crosses between cultivars produce new cultivars, bearing characteristics which are completely different from both parents. Induced mutation has become a proven way for creating variation with a crop cultivar and inducing desired attributes that cannot be found in nature or have been lost during evolution (Booij et al., 1993).

MATERIALS AND METHODS

Off-shoots of three date palm cultivars ('Kulma', 'Mishrig-Wad-laggai' and 'Kursha') were purchased from nurseries in Khartoum State.

Shoot tips with soft inner leaves (leaf bases + axilliary buds), when cultured on Murashige and Skoog medium (Murashige and Skoog, 1962), supplemented with 100 mg/L IBA and 3 mg/L BAP with 1.5 g/L activated charcoal and solidified with 1% agar gave embryogenic cultures after about 4 months under culture. Callus development and multiplication was achieved on a medium with 10 mg/L NAA, 30 mg/L BAP and 1.5 g/L activated charcoal. These cultures were maintained for additional 8 months.

The experiment was carried out on selected cultivars of date palm as different cell lines (Fig. 1).

Shoot tips with soft inner leaves (leaf bases + axillary buds) from the three cultivars were dissected and kept in an antioxidant solution containing 100 mg/L

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L-ascorbic acid.

These were sterilized by immersing in 70% ethanol for 2 min, then in 30% commercial Clorox for 30 min. The explants were washed thoroughly with sterilized distilled water and placed onto the culture media contained in either 100 ml Erlenmeyer flask or 9 cm petri-dish.

Two explants per culture vessel were used (Fig. 2).

The explants were cultured on Murashige and Skoog medium (Murashige and Skoog, 1962), supplemented with 100 mg/L IBA and 3 mg/L BAP with 1.5 g/L activated charcoal, 3% sucrose and solidified with 1% agar. A vitamin mixture of 1 mg/L thiamine and 100 mg/L myoinositol was added to the medium. The cultures were incubated in the dark in a growth room maintained at 25+1°C.

For induction of somatic embryos, MS basal medium supplemented with 10 mg/L NAA, 30 mg/L BAP, 3% sucrose and 1% agar was used.

The tissue culture materials used in anatomical studies were fixed in formalin: glacial acetic acid: 50% ethanol (FAA) 5:5:9, v/v/v (O'Brien and McCully, 1981), embedded in wax, sectioned and stained with safranin/fast-green stain. Sections were examined microscopically and photographed.

RESULTS AND DISCUSSION

Establishment of Callus Cultures

Explants from the three cultivars grown on a medium containing 100 mg/L IBA and 3 mg/L BAP produced white nodular calluses. The callus was maintained for 12 months by repeated subculturing onto a fresh medium of the same composition. The cultures that showed signs of senescence and cease to grow were discarded. Twelve months after callus initiation on medium containing 100 mg/L IBA, only the normal nodular callus was obtained. Selected cultures were subcultured onto media of the same composition and incubated in the dark. White nodular calluses were obtained from shoot tips of the three cultivars (Fig. 3).

Development of Embryos on Media Containing NAA

Attempts to induce somatic embryogenesis in the established callus cultures of the three date palm cultivars were transferred onto media containing 10 mg/L NAA. Only hard compact callus showed differentiation of xylem elements and embryo-like structures (embryoids) with white sheath but no further development of shoot apex.

Anatomical Features of Callus

Few studies were carried out on the anatomy of tissue culture of date palm (Reynolds and Murashige, 1979; Tisserat and De Mason, 1980; Mater, 1989; Al Marrie, 1995; Mohamed, 2000).

Transverse sections of nodular callus revealed that nodules appeared as protrusions on the parent callus and the presence of small cells with prominent nucleus surrounded by larger vacuolated cells resembling parenchyma cells.

A transverse section through a part of the nodular callus revealed a pro-embryonic mass of cells surrounded by larger vacuolated cells. Similar observations were reported by Tisserat and DeMason (1980), Mater (1989) and Mohamed (2000).

Tisserat and DeMason (1980) reported that transfer of callus with meristemoid and early embryoid stages to a medium devoid of auxin (2,4-D) resulted in the formation of plantlets. Mohamed (2000) reported that the nodular callus obtained from the cultures of the leaf bases which showed the presence of meristimatic loci and pro-embryonic mass did not give plantlets upon transfer to a medium containing 0.01 mg/L NAA. In the present study, the nodular callus, obtained from the culture of shoot tips showed the presence of meristemoids and pro-embryonic mass, did not give plantlets upon transfer to a medium containing 10 mg/L NAA.

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Figures

Fig. 1. A) Semi dry fruits of 'Mishrig-wad-laggai'; B) Dry fruits of 'Kursha'.

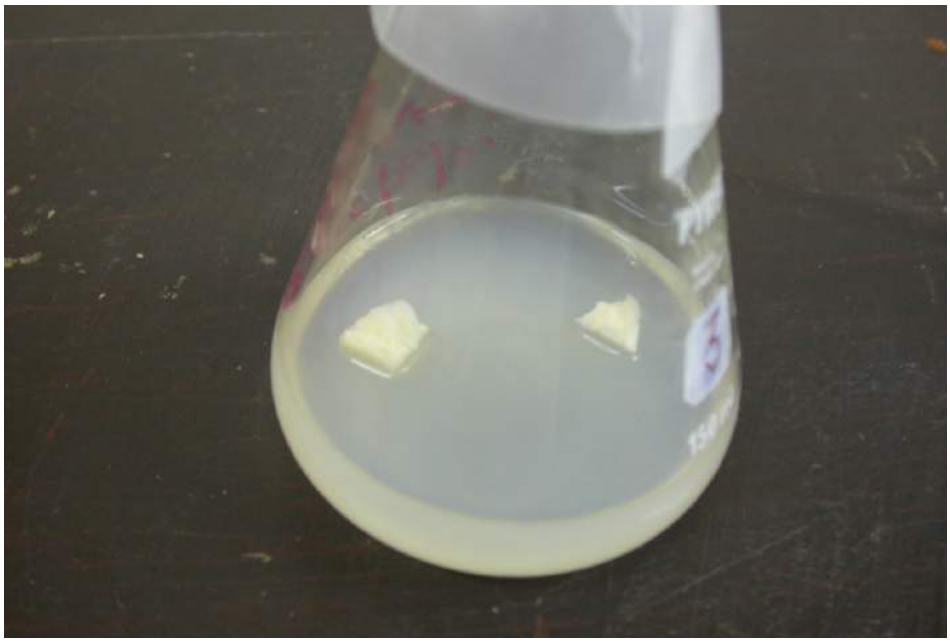

Fig. 2. Date palm explants used for callus initiation on MS medium supplemented with IBA and BAP.

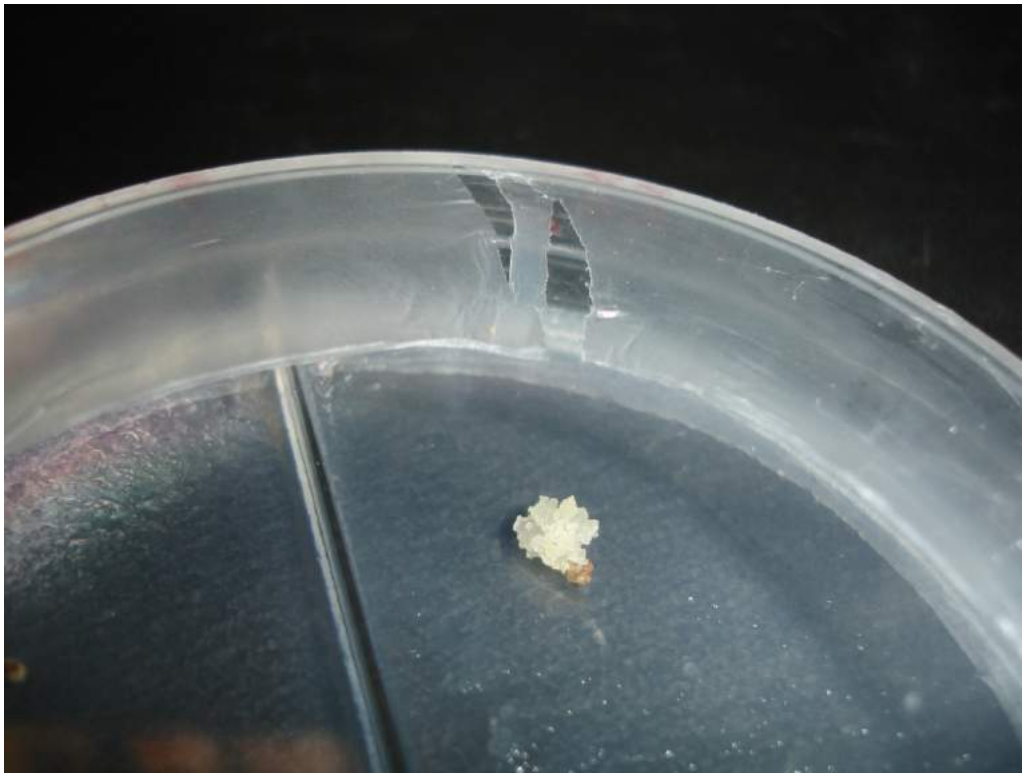

Fig. 3. White nodular callus of date palm after 4 months.

Medium Supplements and Support Matrices for Better In Vitro Growth of Date Palm (*Phoenix dactylifera* L.)

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Keywords: polyurethane foam, support matrix, date palm, tissue culture, micropropagation, activated charcoal, phenol

Abstract

Despite its extensive use as gelling agent for tissue culture media, agar possesses many shortcomings including impurities, scarcity and high cost in local markets and sensitivity to the harsh local environment. In view of the need to find alternative gelling agents or solid support matrices, growth and development of date palm (*Phoenix dactylifera* L.) cultures were evaluated on polyurethane (PU) foam discs in comparison with agar-gelled medium. Incorporating activated charcoal in tissue culture media has been shown to affect growth and development of various organisms. It plays a critical role in the micropropagation of date palms by adsorbing inhibitory compounds in media and decreasing toxic metabolites, phenolic exudation and brown exudate accumulation. However, in some cases activated charcoal adsorbs hormones required for the callus growth and shoot development thereby retarding active growth. A comparative study of in vitro growth responses of two date palm cultivars in the medium containing charcoal and no charcoal showed significant differences in all the growth parameters. Date palm cultures growing on PU foam showed significantly superior rates of shoot multiplication and shoot elongation as compared to cultures in agar-gelled media. The rooting response of cultures on PU foam and agar-gelled media was nearly similar. It is argued that enhanced aeration and better suited physical characteristics of the material may be the reasons for superior performance of PU foam as support matrix in comparison with agar. It is therefore suggested that a polyurethane matrix can be used satisfactorily for micropropagation of date palms. The poor response of date palm cultures in the charcoal containing medium may be attributed to the lowering of pH of medium during autoclaving as reported by earlier workers thereby inhibiting the uptake of required growth regulators. Polyurethane can also be used for special applications where low pH of culture medium is required, and its composition and resulting physical properties may be precisely modified during manufacture to suit specific culture requirements. At the same time the matrix is very cheap as compared to agar even in a single use cycle.

INTRODUCTION

Different types of media supplements and support matrices are used in plant tissue culture to enhance growth and development of explants. Activated charcoal has been used in tissue culture media to improve culture growth and promote morphogenesis in a wide variety of species (Wann et al., 1997). Activated charcoal is often used in plant tissue culture to improve cell growth and development (Pan and van Staden, 1998). It plays a critical role in the micropropagation of date palms by adsorbing inhibitory compounds in media and decreasing toxic metabolites, phenolic exudation and brown exudate accumulation. However, there are reports that, in addition to adsorbing unwanted substances, it may also adsorb needed hormones (Ebert and Taylor, 1990; Ebert et al., 1993; Nissen and Sutter, 1990) vitamins (Weatherhead et al., 1979; Pan and van Staden,

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1998), or metal ions such as Cu^{++} and Zn^{++} (Van Winkle et al., 2003).

In vitro cultures require a surface to grow on. For this reason agar is widely used to gel plant tissue culture media and provide a physical support to the growing cultures. Agar possesses many peculiar properties that suit its use as gelling agent in tissue culture media. It is chemically inert in the medium and is not digested by plant enzymes (Henderson and Kinnersley, 1988). It also forms a uniform gel that remains stable over the range of pH, temperature, and light conditions maintained during incubation.

However, despite the above characteristics, performance of agar as a gelling agent is not always consistent. The quality of agar and corresponding performance of cultures has been found to vary from brand to brand presumably due to varying levels of impurities (Scholten and Pierik, 1998; Nairn et al., 1995; Debergh, 1983). Agar also hinders aeration in the medium and may curtail availability of oxygen to the cultures (Newell et al., 2003; Anon., 1988).

Besides its qualitative shortcomings, agar constitutes the single costliest component of tissue culture media (Puchooa et al., 1999; Bhattacharya et al., 1994). There might be variation in the price of agar of different brands at different locations, but the predominance of its cost would remain overbearing.

Due to its qualitative deficiencies and high cost several attempts have been made to find cheaper alternatives to agar that should put up better or similar performance of cultures as well. Starches from cassava (Maliro and Lameck, 2004; Gerbe and Sathyanarayana, 2001), corn (Puchooa et al., 1999; Hendersen and Kinnersley, 1988), sago (Bhattacharya et al., 1994), and potato (Calleberg et al., 1989), etc. have proved variable efficiencies as gelling agents in culture media. Some gums like gellan gum (Puchooa et al., 1999; Calleberg et al., 1989), gum-katira obtained from bark of *Cochlospermum religiosum* have also been found to impart good gel strength to the medium (Jain and Babbar, 2002). Among other natural products, microcrystal cellulose (Gorivnova et al., 1993), parenchymatic solidifier from apple (Titel et al., 1987), an agar-like polysaccharide obtained from *Pseudomonas* (Kang et al., 1982), husk of *Plantago ovata* seed (Babbar and Jain, 1998; Bhattacharya et al., 1994) have been tried. These materials have ample potential for use as gelling agent and are comparatively cheaper. But their performance has been inconsistent, perhaps due to lack of standardization and presence of impurities.

Synthetic solid matrices offer some distinct advantages over agar and other gelling agents. Their quality and properties can be precisely controlled for consistent performance; they are comparatively very cheap, can be reused and are more convenient to handle. Glass beads and glass beads with filter paper (Puchooa et al., 1999) have shown limited superiority over agar. While some synthetic matrices like glass wool cloth, nylon cloth, polystyrene foam (Bhattacharya et al., 1994), polyester-acetate membrane (Matsumoto and Yamaguchi, 1989), polypropylene membrane (Hew et al., 1990; Tanny et al., 1993; Desamero et al., 1993; Adelberg et al., 1992), and polyurethane foam (Conner and Meredith, 1984) have shown promising results.

This study was designed to test the utility of polyurethane foam as support matrix during two standard procedures of in vitro plant production, viz., callus mediated somatic embryogenesis and shoot bud proliferation in date palm (*Phoenix dactylifera* L.). An attempt was also made to compare the efficacy of media with or without charcoal in the somatic embryogenesis and further growth of date palms.

MATERIALS AND METHODS

Explant Preparation and Sterilization

Two elite cultivars of date palm, ‘Sukkary’ and ‘Mosaifah’ were used for the experiment. Tissue from the apical region of 2-3-year-old offshoot was used as explant. The top 8-10 cm portion was excised and was treated twice with 1.0% solution of sodium hypochlorite for 20 and 10 min respectively followed by treatment with 0.2% solution of mercuric chloride for 5 min. Tween-20 (2-3 drops per 100 ml) was added to both the

sterilizing solutions as surfactant. After rinsing thrice with sterilized distilled water the block was cut into small pieces of 5-8 mm for plating.

Nutrient Media

Medium for date palm cultures consisted of MS (Murashige and Skoog, 1962) salts supplemented with 170 mg L^{-1} mono-sodium phosphate, 200 mg L^{-1} glutamine, 125 mg L^{-1} meso-inositol, 3.0 mg L^{-1} glycine, 0.1 mg L^{-1} thiamine HCl, 1.0 mg L^{-1} pyridoxine HCl, 1.0 mg L^{-1} nicotinic acid and 30 g L^{-1} sucrose.

Plant growth regulators in the media were added according to the genotype and stage of the cultures as described in the following sections. All media were brought to pH of 5.8 before autoclaving. To prevent browning of cultures at initial stages, 1.5 g L^{-1} activated charcoal, 75 mg L^{-1} ascorbic acid and 75 mg L^{-1} citric acid were added to both the media.

Physical Support Systems in the Media

In date palm, callus induction medium was solidified with 8 g L^{-1} agar (Hi Media). At subsequent stages of date palm cultures physical support in the medium was provided by polyurethane (PU) foam discs and agar (8 g L^{-1}) in two separate sets of cultures for the purpose of comparison. 1.5 g L^{-1} activated charcoal was added to the callus induction medium and somatic embryogenesis medium along with agar. Another set of cultures maintained with agar and without charcoal served as control.

Circular discs of polyurethane foam, fitting the inner diameter of the culture jars were cut out from commercially available sheets of 1.5 cm thickness. Material of the foam was confirmed as linear aliphatic polyurethane by mass spectrometry. Density of the foam was estimated to be 0.0142 g cm^{-3} by weight-volume ratio.

The discs were washed with mild detergent, rinsed thoroughly with distilled water, and dried before use. As the discs would gradually absorb medium and sink, enough volume of medium to reach approximately 2/3 the height of the discs was poured in the jars. This level was maintained by 60 ml of medium in the jars used in this study. In the case of agar-gelled media, the same volume was dispensed in each jar.

Culture Procedures

For callus initiation in date palm, small pieces of soft meristematic tissue from the shoot tip zone were implanted on agar-gelled (8 g L^{-1}) modified MS medium containing 450 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 15 μM 6-(γ,γ-dimethylallylamino) purine (2-iP), and 15 μM 6-furfurylaminopurine (kinetin). The explants were sub-cultured in the same medium, after 2 weeks and subsequently, at an interval of 4 weeks. After 4-5 sub-cultures white granular portions of the callus were transferred to another agar-gelled medium containing 15 μM α-naphthalene acetic acid (NAA), 15 μM 2-iP and 15 μM kinetin for the proliferation of embryogenic callus.

After 4 weeks in the proliferation medium granular embryogenic callus was transferred to hormone-free medium for the development and germination of embryos. Further this stage, physical support in the medium was provided by polyurethane foam discs and agar in two separate experimental sets of cultures. Young somatic embryos were sub-cultured twice on this medium at an interval of 4 weeks each before starting collection of data on their rate of multiplication and germination.

For shoot growth, germinating somatic embryos were transferred to fresh hormone-free medium. For root induction, 5-6 cm long shoots were transferred to foam-bearing liquid and corresponding agar-gelled media containing 0.5 μM NAA (Fig. 3). From the callus induction stage to embryo germination stage two sets of cultures; agar with charcoal and agar without charcoal, were maintained.

Data Collection and Analysis

Data was recorded concurrently on cultures on PU foam and in agar-gelled media. Efficiency of the two physical support systems was compared on the basis of parameters

representing growth of somatic embryos, shoots and roots. In the case of date palm, rate of multiplication of somatic embryos was assessed as percent increase in number of these structures in a 4-week period and frequency of germination of somatic embryos was recorded for the same period, while rate of shoot elongation was evaluated as percent gain in shoot length during a 6-week period.

Rooting response in date palm was assessed as days to initiate rooting in 50% cultures. Number of roots per plant was recorded four weeks after initiation. Average length of root after four weeks of initiation was also recorded in date palm cultures. For all parameters, data were recorded on at least 25 cultures in five replicates separated temporally or spatially. The experiment was laid out in completely randomized design and analysis of variance was done by the students 't' test to compare the independent influence of the support matrices and the influence of activated charcoal.

RESULTS

Callusing started early in the explants cultured on media without charcoal, but due to severe browning most of the cultures died (Table 1). Within the next two months somatic embryos started proliferating in granular embryogenic calli. Multiplication of somatic embryos and their rate of germination were found significantly higher in the media without charcoal (Fig. 1). The duration required for different morphogenesis was also found significantly decreased in the media without charcoal (Table 2).

The rate of multiplication of somatic embryos in a 4-week period was significantly higher on PU foam (mean 106%) as compared to 82.6% in agar-gelled medium (Table 3). No significant difference was noticed between responses of the two cultivars on the respective support systems.

Frequency of germination of somatic embryos in 4 weeks was also noted to be superior on PU foam (37.2%) than in agar-gelled media (25.2%). Significantly greater shoot elongation in a 6-week period was recorded on PU foam (55.1%) as compared to agar (36.8%). Germination of somatic embryos on PU foam was better in 'Mosaifah' (38.7%) as compared to 'Sukkary' (35.7%), whereas in agar-gelled medium the two cultivars performed at par with each other, leading to an overall similar response. Elongation of shoots was greater in 'Mosaifah' than in 'Sukkary' on both types of support systems reflecting significantly higher mean for 'Mosaifah (57.7%) as compared to 'sukkary' (34.2%).

Initiation of rooting took nearly the same time both on PU foam (14.8 days) and agar-gelled media (15.4 days) as shown in Table 2. However, 'Sukkary' stroked root significantly earlier on PU foam (14.4 days) as compared to agar (16.7 days), while 'Mosaifah' took the same period of time on both surfaces. In agar-gelled medium, 'Mosaifah' showed roots earlier (14.0 days) than 'Sukkary' (16.7 days).

Means of the two cultivars after 4 weeks of root initiation in 50% of cultures indicate that fewer roots per plant were formed on PU foam matrix (4.1) as compared to agar-gelled medium (5.3). 'Mosaifah' developed more roots (5.9) than 'Sukkary' (4.6) in agar-gelled medium but on PU foam it formed a similar number of roots as 'Sukkary'.

Based on the means, it was noticed that longer roots were formed on PU foam (8.4 cm) than on agar-gelled medium (Fig. 2). Means of the treatments showed greater root length for the cultivar 'Mosaifah' (8.2 cm) because it formed longer roots on both the surfaces as compared to 'Sukkary' (7.0 cm).

DISCUSSION

The study was conducted with two genotypes to take into account the genotypic influence and its interaction with the support matrix and media supplements. Not many cultivars of date palm could be induced to produce callus and somatic embryos because of the comparatively recalcitrant nature of the species. A considerable delay or failure of morphogenesis was observed in the different stages of micropropagation of date palm, when activated charcoal was used. Constantin et al. (1977) observed that the hormones required for the callus growth and shoot development in 'Wincosin-38' tobacco are

adsorbed by activated charcoal, thereby inhibiting callus growth and prohibiting shoot development. In some cases activated charcoal prevented development of callus from embryos in the embryo culture (Nguyen et al., 2007).

During micropropagation the exudation of phenol is very common and it often influences the result. Thomas (2008) compiled some recent reports on application of activated charcoal in plant tissue culture of 105 crops. In 90 cases it was found positive, 12 cases found negative and in 3 cases reported as positive and negative. Two important negative aspects of activated charcoal are catalyzed hydrolysis of sucrose into fructose and glucose (Druart and De Wulf,1993) and drastic reduction of pH after autoclaving (Wann et al., 1997). Phenolic exudation is a matter of serious concern in the micropropagation of date palms. In the explant culture for callus induction incorporation of activated charcoal in the medium is inevitable; otherwise it may lead to considerable loss. On the other hand, as this study indicates, activated charcoal delays morphogenesis and reduces success rate. Alternative use of antioxidants and peroxidases may help to eradicate the problems associated with phenolic exudation in date palm tissue culture and provide the full efficacy of growth hormones and vitamins used in the medium.

In general date palm cultures growing on PU foam showed significantly superior proliferation and germination of somatic embryos and also shoot elongation as compared to cultures in agar-gelled media. Several studies have shown appreciable variation in growth responses of cultures on different solid matrices in comparison with agar. Prasad and Gupta (2006) working with three types of support systems found that shoot production in *Gladiolus* cultures was higher on membrane raft as compared to agar while, shoot elongation was highest on 'duroplast' foam matrix.

Newell et al. (2003) found that compared to agar, rooting response was superior on porous agar, sand, in vitro soil system (IVS) and aerated IVS. They inferred that a higher level of oxygen in aerated systems may have been the cause of early initiation and better growth of roots. Puchooa et al. (1999) have also shown that shoot growth of tobacco cultures in agitated liquid medium allowing enhanced aeration is superior as compared to static liquid medium. Tanny et al. (1994, 1993) have illustrated that cultures growing on a synthetic membrane raft floating on liquid medium put up significantly higher growth as compared to cultures maintained on agar and have suggested aeration and superior water pickup potential of the rafts to be the possible reason for better growth.

In our study somatic embryos and germinated shoots of date palm showing superior growth were placed above the PU foam discs where they received abundant aeration. Gangopadhyay et al. (2004) used coir and 'luffa sponge', the dried vascular net of *Luffa egyptica* fruit for in vitro root induction in *Philodendron* cultures and showed that root initiation was delayed on luffa sponge and coir as compared to agar but the highest number of roots was produced on luffa followed by coir and agar. They have surmised that superior performance of coir and luffa sponge might be due to some purely physical phenomenon. Earlier, they tested coir in comparison with jute and paddy straw as support matrix in media for multiplication of several species and found coir to be the best presumably because of its better water retention capacity (Gangopadhyay et al., 2002). On the other hand, Bhattacharya et al. (1994) experimenting with filter paper, glass wool cloth, nylon cloth and polystyrene foam for micropropagation of chrysanthemum cultures found that generally, these matrices performed at par with agar except that cultures on polystyrene foam produced longer shoots and fewer but longer roots, similar to the findings of the present study. Matsumoto and Yamaguchi (1989) used nonwoven materials like different grades of polyester (100%), polyester-acetate (50:50), acetate (100%), and absorbent cotton as support matrices for culturing protocorm-like bodies (PLBs) of banana. They have noted that gain in fresh weight of PLBs on all nonwoven matrices except a particular type of polyester was significantly superior to agar. Of the physical properties noted, this particular polyester has significantly lower water content (0.6-0.7%) at 20°C-95% RH as compared to all other matrices (10-27%). Tanny et al. (1994, 1993) have suggested that water pickup potential of the material used as support matrix may be one of the physical properties determining availability of water and solvents to the

cultures.

Generally, presence of impurities, inadequate diffusion of nutrients and lack of aeration are arguably considered the major factors responsible for inconsistent and slower growth of cultures on agar (Puchooa et al., 1999; Debergh, 1983). However, there are indications that some physical phenomena may also be playing a role in the variable response of cultures on agar. Like other polysaccharides, agar is a viscoelastic material simultaneously possessing solid and liquid properties with quantifiable modulii of elasticity and viscosity allowing measurement of predominance of solid or liquid characteristics through rheological analysis. On the basis of this analysis Pereira et al. (2007) have shown that elasticity and viscosity differ widely in different brands of agar. Moreover these characteristics change randomly during storage of media and incubation of cultures. Changing modulii influence the interaction between water molecules, media constituents and agar and hence availability of water and nutrition to the cultures. This seems to be a more plausible explanation for inferior performance of agar as compared to the synthetic support system where interaction between water molecules, media components and material of the matrix would remain stable over a long period of time.

Genotypic influence was mild in date palm cultures. It only reflected in greater shoot elongation and root length of cultivar 'Mosaifah'. As an indication of cultivar-matrix interaction, 'Mosaifah' showed higher germination of somatic embryos on PU foam and a greater number of roots in agar. This study shows that PU foam is a satisfactory support material for in vitro cultures. Conner and Meredith (1984) have also successfully used PU foam for monitoring the growth of callus cultures; we suggest that it can be equally beneficial for micro propagation as well. The matrix would be of special use in applications where media are maintained at such pH which would not allow gelling of agar or where in situ change of medium is required. Chemical composition and resulting physical properties of the material may be precisely modified during manufacture to suit specific culture requirements. At the same time the matrix is very cheap as compared to agar even in a single use cycle.

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Tables

Table 1. Response of activated charcoal in the micro-propagation of date palms.

Cultivar	Growth parameters								
	Callus induction (%)			Multiplication of somatic embryos (numbers)			Germination of somatic embryos		
	AC	WAC	t-value	AC	WAC	t-value	AC	WAC	t-value
Sukkary	36.6**	11.71**	21.85	189*	208*	2.73	125**	176**	9.42
Mosaifah	66.6**	29.97**	17.02	450**	512**	3.38	430**	486**	5.28
t-value	21.43	9.14		13.75	57.45		28.9	52.62	

AC - activated charcoal; WAC - without activated charcoal. *Significant at P>0.05; ** significant at p<0.01.

Table 2. Duration of morphogenesis under the influence of activated charcoal.

Cultivar	Number of weeks taken to initiate								
	Callus induction			Somatic embryogenesis			Germination of somatic embryos		
	AC	WAC	t-value	AC	WAC	t-value	AC	WAC	t-value
Sukkary	10	8	0.77	43**	24**	5.89	12	10*	1.55
Mosaifah	8	6	1.22	28**	16**	5.04	9*	6*	2.60
t-value	0.77	1.22		6.3	2.48		1.96	4.9	

AC - activated charcoal; WAC - without activated charcoal. *Significant at P>0.05; ** significant at p<0.01.

Table 3. Shoot growth response of date palm cultivars on PU foam in comparison with agar.

Cultivar	Growth parameters								
	Multiplication of somatic embryos			Germination of somatic embryos			Elongation of shoots		
	Agar (%)	PU foam (%)	t-value	Agar (%)	PU foam (%)	t-value	Agar (%)	PU foam (%)	t-value
Sukkary	88.0**	101.3**	6.02	25.7**	35.7**	4.96	24.0**	44.4**	9.16
Mosaifah	77.3**	110.6**	6.96	24.6**	38.7**	3.50	49.7**	65.8**	5.57
t-value	4.02	2.04		0.55	0.74		9.87	8.37	

*Significant at P>0.05; ** significant at p<0.01.

Table 4. Rooting response of date palm cultivars on PU foam in comparison with agar.

Cultivar	Rooting parameters								
	Days to initiation in 50% cultures			Roots per plant			Mean root length		
	Agar	PU foam	t-value	Agar	PU foam	t-value	Agar	PU foam	t-value
Sukkary	16.7*	14.4	2.19	4.6	4.4	0.55	6.2	7.8	1.93
Mosaifah	14.0	15.2	1.19	5.9*	3.7*	3.24	7.4*	9.1*	2.46
t-value	3.11	0.66		1.93	1.87		2.38	0.97	

*Significant at P>0.05; ** significant at p<0.01.

Figures

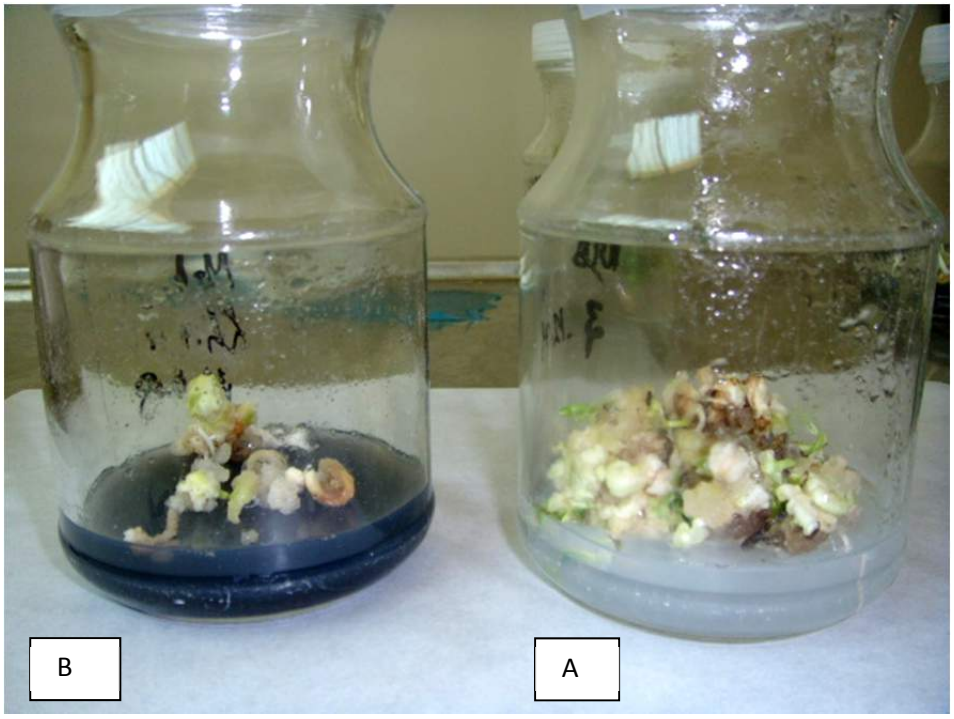

Fig. 1. More somatic embryos and embryo germination on media without charcoal (A) compared to media with charcoal (B).

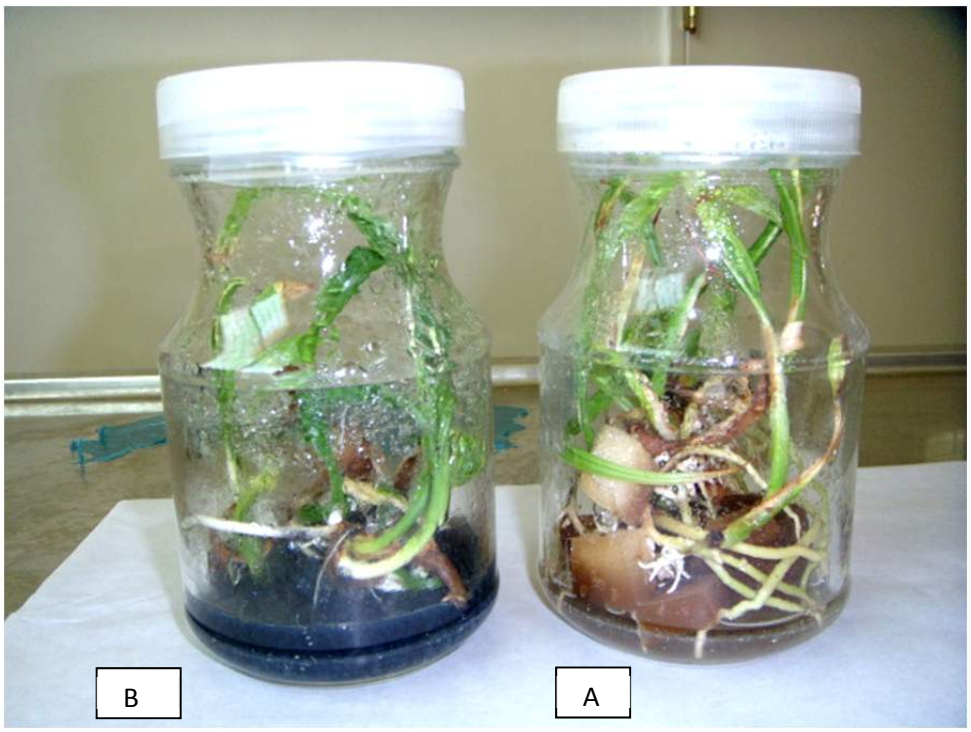

Fig. 2. Profuse and longer rooting in PU foam containing medium (A) compared to agar gelled medium (B).

Micropropagation of Date Palm (*Phoenix dactylifera* L.) Selected Genotypes from Inflorescence Tissues by Using Somatic Embryogenesis Technique

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Keywords: selected genotypes, floral explants, auxins, somatic embryos, vegetative buds

Abstract

Research activities on date palm micropropagation have permitted to multiply a great number of known cultivars worldwide. However, micropropagation of some important rare or selected date palm genotypes is still hampered by the lack of offshoots. To overcome this problem, the use of tissues excised from inflorescences, at their emergence, has permitted to succeed in micro-propagation of many selected genotypes by using the organogenesis technique. However, some selected genotypes are still recalcitrant and it is very difficult to regenerate vegetative buds directly from cultured explants. In order to adapt this technique to such recalcitrant genotypes, the embryogenesis technique was tested to succeed in their multiplication. Three different auxins (2,4-D, Picloram and Dicamba) were tested on callus initiation from inflorescence tissues of a selected date palm genotype INRA-D12. Embryogenic callus was obtained on medium containing 12 mg L^{-1} of Dicamba, 2 mg L^{-1} of BA and 300 mg L^{-1} of activated charcoal. Obtained callus was multiplied on culture media devoid of 2,4-D and activated charcoal. After 4 multiplication cycles, a mixture of somatic embryos and vegetative buds were regenerated. Obtained buds were successfully multiplied and complete plantlets were regenerated. Vegetative buds were similar, both in terms of multiplication rate and behaviour, to those obtained directly from explants excised from date palm shoot tips. Produced plantlets were successfully acclimatized in a greenhouse and are now ready to be transferred to soil for field evaluation. Some vitro-plants will also be reserved for Bayoud resistance tests. In the present protocol, a relatively low auxin concentration and a limited number of callus multiplication cycles were used and this will be more secure concerning true-to-typeness of regenerants.

INTRODUCTION

During the last decade, many papers have been published on date palm tissue culture. The most important works have been done on tissues excised from shoot tips and only few papers were reported on use of floral explants. However, in the case of selected or rare genotypes, there are limited numbers of suckers available to start their multiplication. In such situation, the only way to micro-propagate those genotypes is by using inflorescence tissues. Some valuable works have been published on the use of such plant material (Drira, 1985; Drira and Benbadis, 1985; Loutfi, 1989, 1999; Loutfi and Chlyah, 1998; Abahmane, 1998, 2003, 2005a,b, 2007). The most work on floral tissues has been done on plant material excised from immature inflorescences. However, interesting results have also been undertaken on tissues excised from emerged inflorescences. In the latest situation, inflorescences can be removed without severe damages to the tree. Hence the mother tree is preserved. In Morocco, the use of this technique has as main objective the micro-propagation of some selected genotypes in order to produce enough plantlets to be used in resistance tests against Bayoud disease. In

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fact, the multiplication process has been started in more than ten selected genotypes. The attempts to use only the technique of organogenesis for this purpose encountered some constraints as some genotypes are still recalcitrant. Hence, the use of the somatic embryogenesis technique can lead to overcome this recalcitrance. The objective of this work is to test the efficiency of some auxins to induce callus formation from inflorescence tissues of a selected date palm genotype INRA-D12.

MATERIALS AND METHODS

Plant Material Preparation

Plant material is excised from emerged inflorescences of a selected genotype INRA-D12. This genotype is presumed resistant to Bayoud disease and has good fruit quality but no offshoots are available at its base. The inflorescences were harvested during the flowering period from February to April. The disinfection protocol used was as follows:

1. The spathe is firstly dipped during 10 min in a fungicide solution containing 3 g L^{-1} of Moncozan (mancozeb).
2. Then, it is opened under aseptic conditions and the pedicels were carefully collected and dipped during 15 min in a solution of sodium hypochlorite 50% (Fig. 1a).
3. Finally the pedicels were washed three times with sterile distilled water before transferring on culture media.
4. Plant material can be soaked in an antioxidant solution (ascorbic acid: 100 mg L^{-1} and citric acid: 150 mg L^{-1}) until its inoculation on culture media.

Segments of pedicels (1.5-2 cm in length) with at least 2 flowers were used as explants (Fig. 1b).

Culture Medium

Culture medium used in this experiment consisted of Gamborg and Eveleigh (1968) basal medium as modified by Gresshoff and Doy (1972) for macro-elements, Gamborg and Eveleigh (1968) basal medium for microelements and iron sources of Murashige and Skoog (1962) basal medium. Beyond these mineral salts, the following compounds were incorporated in the culture medium (mg L^{-1}): myo-inositol (100), adenine (25), tyrosine (250), glycine (2), biotin (0.01), thiamin-HCl (1), nicotinic acid (0.3), sucrose (40000), agar (8000) and PVP-40 (2000).

Three different auxins (2,4-D, Picloram and Dicamba) at 12 mg L^{-1} coupled with 2 mg L^{-1} of BA and 300 mg L^{-1} of activated charcoal were tested on callus induction. The medium was adjusted to pH 5.7 after addition of all compounds. The cultures were incubated in darkness at 27±1°C during the illuminated period and 22±1°C during the dark period. The photoperiod was 16 hours per day. Transfers to fresh media were done at one month intervals. Culture medium was dispensed in test tubes (25×150 mm) at 15 ml per tube before autoclaving during 20 min under 1 bar pressure.

Statistical Analysis

The experiment was arranged in a factorial completely randomized design. Analysis of variance was performed using statistical software "SYSTAT" and the means were compared according to the Newman and Keuls test (Dagnelie, 1980) at 5%. Each treatment was repeated three times and contained 90 explants. Two explants were inoculated per test tube.

RESULTS AND DISCUSSION

Callus Induction

The effect of three different auxins (2,4-D, Picloram and Dicamba) was tested on floral explants excised from emerged inflorescence of selected genotype INRA-D12. The first common reaction on the three culture media was root formation. These roots take

place from the axils of flowers and their development was more pronounced on culture medium containing Dicamba. Statistical analysis showed significant differences between tested media. The second spectacular reaction was growth of carpels. In fact, some explants have shown one, two or three carpels development. In some cases, development of more than three carpels has been observed. Statistical analysis did not show any significant differences between tested media. The two previous reactions took place after two months of culture and reached the maximum in the third month. Similar results about root and carpels growth were reported in the literature when culture media, containing high levels of auxins, were used (Drira and Benbadis, 1985; Loutfi, 1999; Abahmane, 2005a).

Embryogenic callus was obtained on culture medium containing 12 mg L^{-1} of Dicamba, 2 mg L^{-1} of BA and 300 mg L^{-1} of activated charcoal. The formed callus was white coloured and originated from outer floral parts. The reported literature on somatic embryogenesis in date palm has shown that floral explants excised from immature inflorescences needed low concentrations of auxins for callus induction. In fact, Drira and Benbadis (1985) have reported callus formation on culture medium containing (mg L^{-1}) IBA (0.5), 2,4-D (0.3-0.5) and BA (0.2) when very young inflorescence tissues were used. However, floral tissues excised from emerged inflorescence required high auxin concentrations for callus initiation. Hence, Loutfi (1989) reported that 100 mg L^{-1} of 2,4-D was required for callus induction from emerged inflorescence tissues of 'Jihel' and 'Iklane' Moroccan cultivars. In addition, Karun et al. (2004) reported that use of 2,4-D at 68 µM did not succeed to induce callus formation from immature inflorescence of the monoecious palm *Areca catechu* L. In contrast, they obtained embryogenic callus with Picloram at a concentration of 200 µM.

Callus Multiplication and Regeneration

The obtained callus was transferred on culture medium containing MS basal salts at half strength supplemented with NAA (0.2 mg L^{-1}), 2iP (0.1 mg L^{-1}) without activated charcoal. At the beginning of transfer, the callus multiplication was too slow but after the second transfer, the rate of multiplication was better. After four transfers on this culture medium, we noticed a beginning of regeneration of a mixture of vegetative buds and somatic embryos (Fig. 1c). The same results have been reported by Ferry et al. (2000) on some Spanish cultivars. They reported an embryogenesis organogenesis mixed system for in vitro date palm propagation. In addition, Loutfi and Chlyah (1998) have reported development of shoots from calli after their transfer on culture medium containing 0.5 mg L^{-1} of NAA, 2 mg L^{-1} of BA and 1 mg L^{-1} of 2-iP.

Regenerated vegetative buds were collected and transferred on multiplication culture medium. At this step, only regenerated buds were multiplied instead of callus multiplication.

Shoot Multiplication

Regenerated buds were transferred on culture medium containing half strength of MS basal salts supplemented with low concentrations of growth regulators as previously developed for regenerated buds by organogenesis technique (Abahmane, 2005b). At the beginning of the shoot multiplication stage, buds growth was slow and the multiplication rate was no more than 1.2. After the second subculture, this situation has completely changed. In fact, shoot multiplication was satisfactory and reached a rate of 1.9 every 4 to 5 weeks. Shoots behaviour was also similar to those regenerated directly from inoculated explants by organogenesis technique (Fig. 1d).

The obtained results were in accordance with those reported by Al Khateeb (2006) on shoot multiplication of 'Sukry' date palm. He stated that low hormone concentrations promoted formation of new buds while high concentrations resulted in abnormal growth and no sign of budding or shoot formation was observed. According to his study, the best combination that gives a good multiplication rate contained no more than 0.3 mg of auxins and 0.4 mg of cytokinins.

Shoot Elongation and Rooting

In order to produce complete plantlets, shoots in the multiplication stage were transferred on culture medium containing MS salts at half strength supplemented with 0.1 or 0.2 mg L^{-1} of NAA (Fig. 1d). At this step, bud's leaves were left without cutting to promote their elongation. By doing that, clusters of small plantlets were formed and were separated later. On the previous medium, elongated shoots produced also many roots. After six transfers on the same medium, well formed plantlets were obtained and were ready to be transferred to the acclimatization stage (Fig. 1e).

Plant Acclimatization

The obtained plantlets in the previous stage were transferred in plastic bags under greenhouse. The substrate consisted in a mixture of peat moss and small gravel at equal volume (v/v). The temperature under greenhouse was maintained at 28±2°C and the relative humidity at 70%. To upgrade relative humidity around the newly transferred plantlets, a micro-tunnel covered by transparent plastic was used and consequently relative humidity was up to 90%. Every 2 to 3 days, plantlets were sprayed with a fungicide (Pelt 44) to prevent crown and leaves rots. Under these conditions, plantlets having 2 to 3 leaves, a well formed and closed crown and 3 to 4 leaves showed a high percentage of surviving of about 90%. After six months, more than one hundred of well acclimatized plantlets were obtained and are now under hardening conditions (Fig. 1f).

CONCLUSIONS

An efficient regeneration protocol for rare and selected genotypes with limited numbers of offshoots has been established. The protocol starts from floral tissues excised from emerged inflorescences. By using this technique, the mother tree can be multiplied without severe damages.

During the experimentation, the use of Dicamba at 12 mg L^{-1} was more effective in inducing callus formation, in the selected genotype INRA-D12, compared to 2,4-D and Picloram used at the same concentration. The obtained embryogeneic callus was multiplied on culture media devoid of 2,4-D and activated charcoal. After a limited number of subcultures, the calli were transferred on regeneration medium with low hormonal concentrations. A mixture of somatic embryos and vegetative buds were regenerated. Obtained buds were then multiplied instead of callus multiplication. Vegetative buds were similar, both in terms of multiplication rate and behaviour, to those obtained directly from explants excised from date palm shoot tips. Complete plantlets were produced and successfully acclimatized under greenhouse.

Produced plantlets will be used to confirm Bayoud resistance of the multiplied genotype (INRA-D12). The remaining plantlets will be transferred to soil at the INRA experimental station for field evaluation.

In the described protocols, a limited number of callus multiplication cycles and relatively low auxin concentrations were used and this will be more secure concerning true-to-typeness of the regenerated plants. This protocol is actually used for micropropagation of other selected genotypes that are recalcitrant in the organogenesis technique.

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Figures

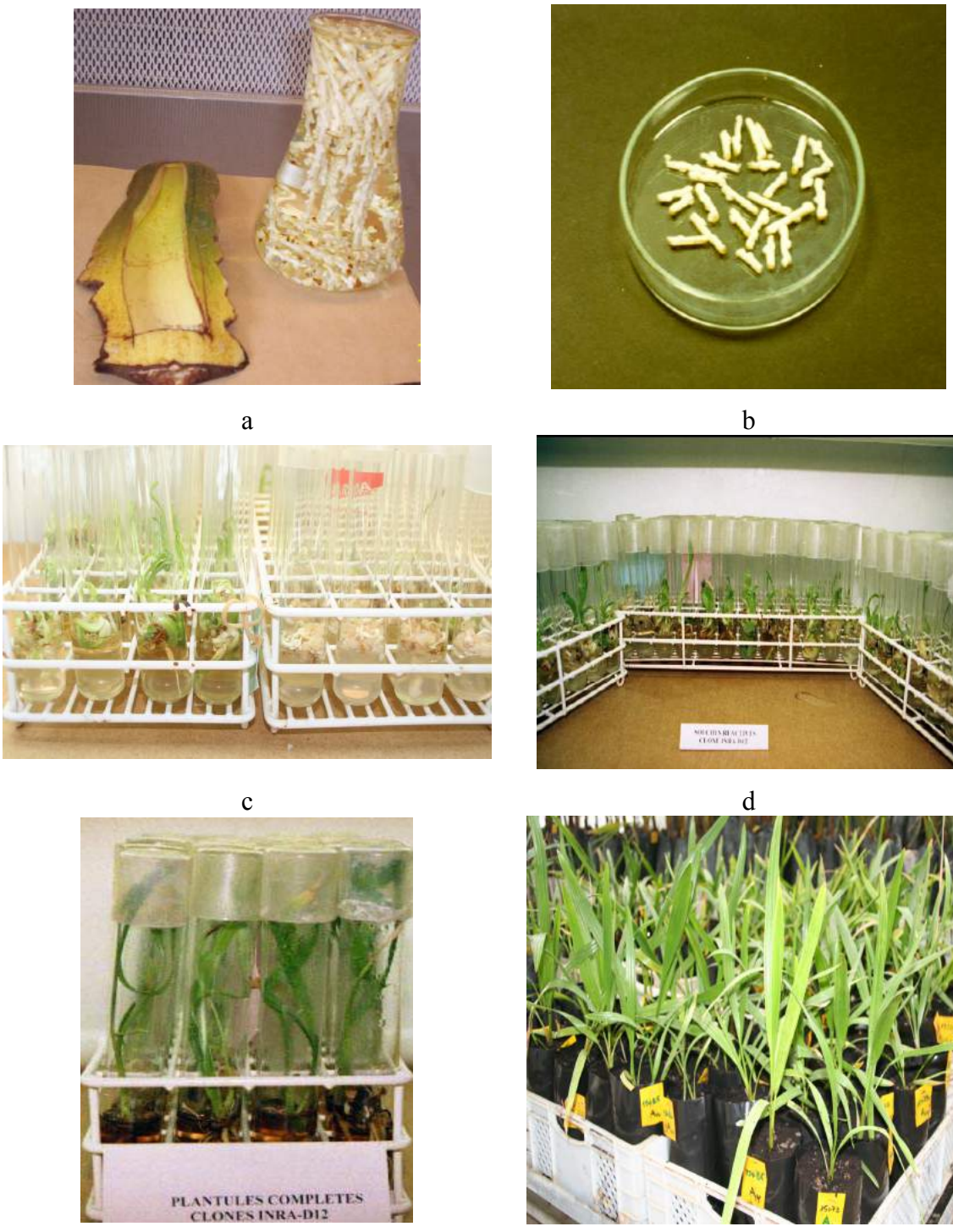

Fig. 1. Use of emerged inflorescence tissues for date palm micropropagation. a) Plant material extraction and disinfection techniques; b) explants ready to be inoculated on culture media; c) regenerated somatic embryos and vegetative buds from embryogenic callus; d) shoots at the elongation stage; e) produced plantlets ready to be transferred to soil; f) well acclimatized plants at the hardening stage.

Positive Effects of Arbuscular Mycorrhizal Fungi on Biomass Production, Nutrient Status and Water Relations in Date Palm Seedlings under Water Deficiency

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Keywords: *Phoenix dactylifera*, water stress, water potential, relative water content, nutrients concentrations, AMF

Abstract

The contribution of arbuscular mycorrhizal fungi (AMF) *Glomus clarum* (GC), *Glomus deserticola* (GD), *Glomus monosporus* (GMO) and Complex Aoufous (CAF) to biomass production and mineral nutrients acquisition was investigated in date palm seedlings subjected to water deficit under greenhouse conditions. Two watering treatments (75 and 25% of field capacity) were applied 4 months (WT1) or 8 months (WT2) after AMF inoculation. Results indicated that water deficiency, WT1 and WT2, reduced shoot dry weight and plant concentrations of P, K, Ca, Mg and Mn both in mycorrhizal and non-mycorrhizal plants. However, under both water stress regimes, AMF plants showed higher nutrient level than non-AMF plants. Highest biomass and nutrient concentrations were recorded in plants inoculated with CAF both in WT1 and WT2. Water relations related to drought tolerance were investigated in non-inoculated and CAF inoculated date palm seedlings. Results showed that under water deficit (25% FC), CAF-inoculated seedlings maintained their RWC content and water potential at a higher level and their cell modulus of elasticity and symplastic water content at a lower level than non-mycorrhized plants. AMF seedlings showed higher (less negative) $\Psi\pi_0$ and Ψ_π^{100} than non-AMF seedlings both under well watered (75% FC) and water stress (25% FC) conditions. These data suggest that the strategies developed by mycorrhizal date palm seedlings under prolonged water deficit were related to modifications in cell wall elasticity and redistribution of water between apoplastic and symplastic compartments.

INTRODUCTION

In the arid and semi-arid areas date palm trees (*Phoenix dactylifera* L.) are considered crucial to the ecosystem as they protect the surrounded vegetation against desert influences and provide adequate microclimate to the under storey crops. However, drought stress is considered to be one of the most important abiotic factors limiting considerably date palm growth and yield (Haddouch, 1997). Arbuscular mycorrhizal fungi (AMF) can form a symbiotic association with the vast majority of land plants including those of the arid areas (Brundrett, 1991). Once established, AMF symbiosis is known to benefit mineral nutrition and to provide enhanced water relations thereby enhancing host plant protection against the detrimental effects of drought (Bethlenfalvay et al., 1988; Davies et al., 1992; Ruiz-Lozano et al., 1996). In date palm, investigations on found AMF symbiosis to promote palm growth and nutrients uptake and was recognized

as positively significant for their establishment and survival (Khaliel and Abou-Heilah, 1985; Oiahabi, 1991; Al-Whaibi and Khaliel, 1994; Morte and Honrubia, 2002; Meddich et al., 2004). The objective of the present study is to evaluate the effect of AMF on biomass production, nutrients status and water relations in date palm seedlings under water deficiency.

MATERIALS AND METHODS

Seeds of date palm, collected from the date palm population of Marrakech, were pre-germinated on wet sand for two weeks. Young germinations were transferred in pots containing 1 kg of sterilized soil (collected from a date palm grove) without or with inoculum of arbuscular mycorrhizal fungi (AMF) and grown under greenhouse conditions. Three *Glomus* species - *Glomus clarum* (GC), *Glomus deserticola* (GD) and *Glomus monosporus* (GMO) - supplied by the Estación Experimental del Zaidín (Granada, Spain) and a locale strain Complex Aoufous (CAF) isolated from the soil of Tafilalet in the South-EST of Morocco were assessed. AMF inoculum consisted of 10 g of a mixture of rhizospheric soil from trap cultures containing spores, hyphae and mycorrhizal root fragments of the corresponding AMF. The same amount of autoclaved mixture of inoculum was added to non-inoculated plants. Water stress treatments began 4 months (WT1) or 8 months (WT2) after AMF inoculation. Water stress treatments consisted of two watering regimes: 75% of field capacity (75% FC) and 25% field capacity (25% FC). The experiment was arranged in a completely randomized block design. Each treatment was replicated 20 times. 30 days after water stress application, shoot dry weight (SDW) was recorded. Dried plant materials were ashed in a furnace for 6 h at 500°C. The ash was dissolved in 20% sulfuric acid, diluted in distilled water and filtered through a whatman filter paper for P, Ca, Na, K, Mg, Cu and Mn analysis. Water relations related to drought tolerance were assessed in non-inoculated and CAF inoculated seedlings. Relative water content (RWC) was calculated using the technique described by Turner (1981). Leaf water potential (Ψ_w) was measured by the method of chamber pressure developed by Scholander et al. (1965). Osmotic potential at full turgor (Ψ_π^{100}), osmotic potential at turgor loss ($\Psi\pi_0$), symplastic water (W_S), and cell elasticity modulus (ξ) were obtained from the pressure-volume curve method (Tyree and Hammel, 1972). All data were analyzed statistically by an analysis of variance using ANOVA modules of the Statistica software program (Statsoft, 1995). Mean comparisons were conducted using Newman-Keuls test at $P<0.05$.

RESULTS

After watering treatments applied four months (WT1) or eight months (WT2) after AMF inoculation, mycorrhizal plants showed higher biomass (Fig. 1) and tissue nutrients concentrations (Tables 1 and 2) than non-AMF plants both under favourable (75% FC) and limiting (25% FC) water conditions. Under well watered conditions, compared to non-AM plants, AM seedlings biomass increase varied from 35 to 75% and from 133 to 161% respectively for WT1 (Fig. 1A) and WT2 (Fig. 1B). In addition, AMF plants nutrient concentrations increased significantly only for P and K in WT1 (Table 1) but for all nutrients in WT2 (Table 2). Under water deficiency, mycorrhizal seedlings exhibit significantly higher biomass and higher P, K, Mg, Ca and Mn concentration than non-inoculated plants both in WT1 (Table 1) and WT2 (Table 2). The effect of AMF on Na and Cu concentration was only significant in WT2 (Table 2). The highest biomass and nutrient concentrations were recorded in plants inoculated with CAF both in WT1 and WT2. Effect of AMF colonization on water relations related to drought tolerance was investigated in date palm seedlings inoculated with CAF compared to non-AM plants. Water relations parameters (RWC, Ψ_w, Ψ_π^{100}, $\Psi\pi_0$, W_S and ξ) were significantly affected by water deficiency (Table 3). RWC and Ψ_w were markedly decreased by water stress in non-AM than in CAF seedlings. Indeed, RWC was 4% higher and Ψ_w was 10% higher in CAF seedlings than in non-AM seedlings (Table 3). However, W_S and ξ of non-AM plants were respectively 1.5- and 2.5-fold higher than in CAF plants. CAF seedlings

showed higher (less negative) $\Psi\pi_0$ and Ψ_π^{100} than non-AM seedlings both under well watered (75% FC) and water stress (25% FC) conditions (Table 3).

DISCUSSION

Arbuscular mycorrhizal fungi had positive effects on biomass production and nutrients concentrations analyzed in date palm seedlings four months (Table 1) and eight months (Table 2) after AMF inoculation. Indeed, shoot dry weight and tissue nutrients concentrations were higher in AMF inoculated than in non-AMF plants. Water stress applied either four months (WT1) or eight months (WT2) after AFM inoculation induced a significant decrease in biomass (Fig. 1A and B) and plant nutrient concentrations (Tables 1 and 2). This negative effect was more pronounced in non-AMF than in AMF plants. Hence, seedlings inoculated with CAF were less affected by water deficiency. Results were in accord with the finding that has been reported for other plant species (Ruiz-Lozano and Azcon, 1996; Kaya et al., 2003; Wu and Xia, 2005). The AMF positive effect was likely attributed to the improvement of nutrient assimilation and water uptake (Sweatt and Davies, 1984; Bethlenfalvay et al., 1988) and to the increase of root length density (Bryla and Duniway, 1997). In the present study, water stressed AMF seedlings maintained better water relations in terms of relative water content, water potential and turgid potential as compared to non-AMF seedlings (Table 3). Similar results were reported by Porcel and Ruiz-Lozano (2004) who showed that leaf water potential determined at the end of the drought stress period decreased larger in non-AM plants than in AM plants. Maintenance of favourable plant water relations is vital for the development of drought adaptation in crop plants (Blum, 1996; Passioura, 2002). The higher RWC and Ψ_w and the lower W_S and ξ of AM plants, compared with non-AM plants, were propitious to moving liquid water through the plants to the evaporating surfaces in the leaves (Nelsen and Safir, 1982). Also, the difference between Ψ_π at full and zero turgid for a given tissue tended to be smaller when cells have more rigid walls. The reverse was observed in mycorrhizal date palm seedlings. Although low ξ values (corresponding to flexible cell walls) have been correlated with drought-adaptation and may provide cells with a high resistance to water stress (Zimmermann, 1978; Robichaux, 1985; Goicoechea et al., 1995).

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Tables

Table 1. Plant nutrient concentrations of mycorrhizal and non-inoculated (NM) date palm seedlings subjected to two watering treatments, 75% field capacity (75% FC) and 25% field capacity (25% FC), four months after AMF inoculation.

Water regime	AMF inoculant	Nutrient concentrations (mg/plant)						
		P	Ca	Mg	Na	K	Cu	Mn
75% FC	GC	82.6a	7.3a	16.2a	1.5a	20.0a	0.3a	0.08a
	CAF	60.8b	8.8a	19.4a	1.0a	24.4a	0.3a	0.08a
	GMO	45.5bc	6.0a	12.6a	1.1a	21.4a	0.2ab	0.05a
	GD	53.0b	8.1a	10.9a	1.2a	20.5a	0.2ab	0.05a
	NM	16.9d	5.0a	7.2a	0.5a	13.6a	0.1b	0.04a
25% FC	GC	44.0b	7.6a	16.1a	1.1ab	12.0bc	0.24a	0.023a
	CAF	57.8b	6.0a	21.6a	1.8a	19.7a	0.25a	0.023a
	GMO	36.3c	6.2a	12.9a	0.8b	13.7b	0.20a	0.021a
	GD	10.9de	4.7ab	5.2b	0.7b	7.7c	0.10a	0.016ab
	NM	16.2d	2.3b	3.9b	0.7b	6.9c	0.13a	0.009b

The same letter within each column indicates no significant difference among treatments (P=0.05).

Table 2. Plant nutrient concentrations of mycorrhizal and non-inoculated (NM) date palm seedlings subjected to two watering treatments, 75% field capacity (75% FC) and 25% field capacity (25% FC), eight months after AMF inoculation.

Water regime	AMF inoculant	Nutrient concentrations (mg/plant)						
		P	Ca	Mg	Na	K	Cu	Mn
75% FC	GC	103.9ab	35.5a	42.2ab	4.3ab	24.3ab	0.4ab	0.08ab
	CAF	93.2ab	24.3ab	60.2ab	5.0ab	43.9ab	0.5ab	0.11ab
	GMO	170.5a	18.0ab	91.7a	6.8a	73.9a	0.8a	0.18a
	GD	104.4ab	28.6ab	60.5ab	6.8a	53.5ab	0.5ab	0.10ab
	NM	34.0b	11.2b	22.2b	1.9b	16.3b	0.2b	0.03b
25% FC	GC	69.0b	23.1ab	34.0a	4.0a	18.5ab	0.3a	0.04b
	CAF	108.9ab	15.4b	40.6a	3.3a	34.5a	0.4a	0.06a
	GMO	46.2b	32.2a	30.3a	3.3a	36.1a	0.3a	0.07a
	GD	50.7b	9.7b	21.1ab	2.8a	19.1ab	0.2a	0.02b
	NM	16.5b	10.4b	7.9b	2.1a	10.4b	0.1a	0.01b

The same letter within each column indicates no significant difference among treatments (P = 0.05)

Table 3. Relative water content (RWC), leaf water potential (Ψ_w), symplastic water (W_S), osmotic potential at full turgor (Ψ_π^{100}), osmotic potential at turgor loss ($\Psi\pi_0$) and cell modulus of elasticity (ξ) of CAF inoculated (AM) and non-inoculated (Non-AM) date palm seedlings subjected to two watering treatments 75% field capacity (75% FC) and 25% field capacity (25% FC).

Water regime	AMF status	RWC (%)	Ψ_w (Mpa)	W_S	Ψ_π^{100} (Mpa)	Ψ_π^{0} (Mpa)	ξ
75% FC	Non-AM	98.62a	-30.5a	5.6a	-4.8b	-15.3b	1.77b
	AM	99.11a	-27.2b	5.3b	-6.9a	-25.0a	3.52a
25% FC	Non-AM	93.48b	-33.6b	2.5a	-20.0a	-28.6a	1.32a
	AM	96.96a	-37.0a	1.7b	-13.3b	-25.0b	0.54b

The same letter within each column indicates no significant difference among treatments (P=0.05).

Figures

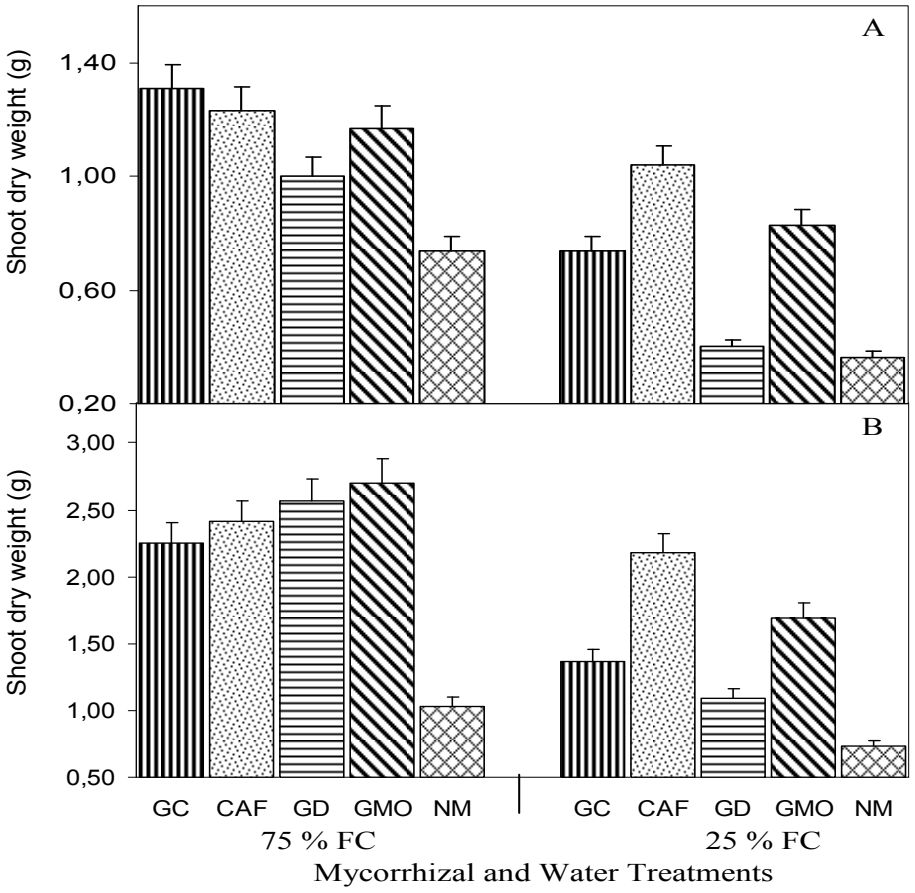

Fig. 1. Shoot dry weight of mycorrhized (AM) and non-mycorrhized (Non-AM) date palm seedlings subjected to two watering treatments, 75% field capacity (75% FC) and 25% field capacity (25% FC), four months (A) or eight months (B) after AMF inoculation.

Effect of Exogenous Indole Butyric Acid (IBA) on Rooting and Leaf Growth of Small Date Palm Offshoots (*Phoenix dactylifera* L.) Derived from Adult Vitroplants of 'Najda' Cultivar

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Keywords: date palm, offshoots, IBA, root formation, leaf growth

Abstract

'Najda' (INRA-3014) is one of the interesting date palm genotypes that were selected for several highly desirable characters, micro-propagated and distributed as vitroplants to date palm growers in Morocco. However, it has been frequently found that adult vitroplants of this cultivar produce lots of offshoots, most of which are high offshoots. Removal of these offshoots is essential for the mother plant and the removed offshoots can be used as an additional source of high quality planting material for the intensification of date palm cultivation in Morocco. Promoting root formation on these offshoots weighing less than 8 kg is therefore essential. The present study was conducted to evaluate the effect of exogenous indole butyric acid (IBA) on root formation and leaf growth of 'Najda' small offshoots. Removed offshoots from adult 'Najda' vitroplants grown in the Zagora Date Palm Experimental Station were separated in 4 weight groups (0-2, 2-4, 4-6 and 6-8 kg). Prior to planting in pots, offshoots were dipped for 15 min in 4 IBA solutions (2, 5, 10 and 15 mg L^{-1}). Results showed that exogenous IBA induced significant rooting of 'Najda' offshoots. More than 96% of offshoots rooted if treated with 10 or 15 mg L^{-1} IBA solutions, while only 50% rooting was obtained with 2 mg L^{-1} IBA treated and control offshoots. These rooted offshoots produced an average of 12 visible roots per offshoot. Furthermore, 90% of offshoots of less than 2 kg rooted well if treated with 10 or 15 mg L^{-1} IBA solutions. Offshoots' rooting was also accompanied by a good leaf growth. During this 8-months experiment, offshoots treated with 10 or 15 mg L^{-1} IBA solutions produced an average of 4 new leaves, and an increment of 9 cm in the central leaf length. Based on these results, it seems reasonable to conclude that treatment with 10 or 15 mg L^{-1} IBA solutions, allows a good rooting of small high offshoots of 'Najda'.

INTRODUCTION

It has been found that date palm vitroplants distributed and planted in the field (Under the National Plan for Reconstruction of the Moroccan palm grove), frequently tend to produce many offshoots, most of which are high offshoots (Fig. 1). Our field surveys in 2007-2008 showed that adult date palm vitroplants about 10 years old may have an average of 20 to 30 offshoots. This high ability of vitroplants to produce many offshoots might be due to the residual effect of cytokinins hormones used during the in vitro propagation process.

To lighten the palms from this heavy load, farmers often remove these offshoots and throw them thereafter, causing a huge loss in expensive plant material usually derived from selected genotypes. Indeed, these offshoots should be used as additional planting material for the reconstitution of the Moroccan palm groves, especially when it comes to valuable genotypes as the 'Najda' date palm cultivar. Indeed, it has often been

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recommended to researchers to invest in this aspect in order to develop a technology to take advantage of the large number of offshoots found in adult date palm vitroplants in the field.

Date palm propagation through offshoots planting is a common method still used by most growers. However, its success remains limited to the use of offshoots weighing between 7 and 15 kg (Perreau-Leroy, 1958; Toutain, 1972; Saaidi et al., 1979). Successful planting from small offshoots weighing less than 7 kg (such as those produced by adult vitroplants), strongly depends on their successful rooting in nurseries prior to their final planting in the field. Promotion of root formation on these offshoots could be insured through the exogenous input of auxin hormones.

It is widely known that auxins play a key role in rooting stimulation and plant propagation. This role of auxins has been reported by several authors, namely in cuttings rooting of several species such as almond (Caboni et al., 1997) and eucalyptus (Fett-Neto et al., 2001), as well as in in vitro rooting of micro propagated avocado and kiwi plantlets (Zirari, 1990; Zirari and Lionakis, 1994). Indole butyric acid (IBA) is currently the most widely used hormone for rooting enhancement, due to its proven ability to promote root initiation (Weismann et al., 1988), its low toxicity and high stability comparison with naphthalene acetic acid (NAA) and indole 3 acetic acid (IAA) (Blazich, 1988; Hartmann et al., 1990).

However, few results exist on auxins effects on rooting improvement of date palm offshoots. In recent decades, the rooting ability of date palm high offshoots has been substantially improved by using the mist sytem (BenAbdelah, 1990; Qaddoury and Amssa, 2004).

The present investigation was therefore undertaken, on the one hand to demonstrate the role of IBA in rooting of date palm offshoots, and on the other hand to develop a rooting technique for small offshoots derived from adult vitroplants of the 'Najda' date palm cultivar.

MATERIALS AND METHODS

Offshoots weighing less than 8 kg were collected from adult 'Najda' vitroplants planted at the INRA Date Palm Experimental Station of Zagora, Morocco.

Offshoot removal was carefully made by skilled laborers, who once the offshoot is free they cut up the leaves, removing about the upper half of their length. The greater width of the bases of the removed offshoots was then marked, and we noted the distance between this marked point and the top of the central leaf, the number of existing palms as well as the weight of each offshoot. All offshoots were subsequently grouped into the following 4 weight classes: 0-2, 2-4, 4-6 and 6-8 kg (Fig. 2). Prior to planting in pots, the bases of these offshoots were dipped for 15 min in the following 4 IBA solutions: 0 (distilled water, control), 2, 5, 10 and 15 mg L^{-1}. Offshoots were subsequently planted the same day of their removal, in standard black PVC containers, containing a potting mixture of 1/3 soil + 1/3 fine sand + 1/3 peat. All the containers with their offshoots were placed in a shade structure, and arranged in a randomized complete block design containing 3 blocks and 3 offshoots per experimental unit (Fig. 3).

Immediately after planting, offshoots were thoroughly hand irrigated to bring the potting mixture to container capacity. During the 8 months of the study, all offshoots were irrigated once every two days during the period from April to September, and once a week during October and November. This schedule has helped maintain the substrate at or near to its water holding capacity. All offshoots were monthly treated with a 100 mg/hl benomyl solution. After two months of study, offshoots received a 300 ppm nitrogen solution. After 8 months, offshoots were removed from their containers, and we recorded their number of new leaves and their central leaf extension. Using a moderate water jet to remove the potting mixture, we recorded the number of rooted offshoots and the number of produced roots per rooted offshoots.

Data statistical analysis was performed using ANOVA test at 5%, with two classification criteria, while the means comparison was performed using Fisher's least

significant difference at 5%.

RESULTS

This investigation has revealed that the rooting of small offshoots removed from adult 'Najda' date palm vitroplants, can be significantly improved by the use of exogenous IBA hormone. The analysis of Table 1 shows that over 96% of offshoots treated with 10 or 15 mg L^{-1} of IBA solutions have produced roots, while only 35.3% of the control offshoots have produced roots (Fig. 4). In addition, IBA treated offshoots produced more roots than those of the control. The maximum mean number of roots produced per offshoots (10.88 and 14.11) were observed in offshoots treated with 10 mg L^{-1} IBA and those treated with 15 mg L^{-1} IBA, however, with no significant difference between them. The untreated offshoots (control) gave few roots (an average of 3.75 roots per rooted offshoots).

The differences obtained in terms of rooting percentage and number of roots produced per offshoots, were followed by significant differences in the vegetative growth (number of produced palms and central leaf extension) (Fig. 5). A net extension of the central leaf was obtained on offshoots treated with 5, 10 and 15 mg L^{-1} IBA solutions, however, with no significant difference between them. With a concentration of 15 mg L^{-1} IBA, an elongation of 9 cm was achieved by the central palm that is twice the leaf elongation obtained on the untreated offshoots. Concerning the number of produced leaves, there were no significant differences between the treated and the untreated offshoots.

The IBA effect was different according to the weight of the offshoot (Fig. 6). The lower the weight of the offshoot, the more difficult was its rooting. The best rooting percentages were obtained with offshoots of the weight classes 4-6 and 6-8 kg. Offshoots of the weight classes 0-2 and 2-4 kg produced the lowest percentages of rooted offshoots, especially when untreated with IBA or treated with low IBA doses (2 and 5 mg L^{-1}). When offshoots were soaked in solutions of 10 and 15 mg L^{-1} of IBA, the percentages of rooted offshoots reached over 90% for all weight classes used in the study. Therefore, after 8 months of the present study, even small offshoots of less than 2 kg could reach 90% rooting, if previously treated with IBA solutions at 10 or 15 mg L^{-1} doses.

DISCUSSION

In the present study, rooting enhancement of date palm offshoots collected from 'Najda' was achieved by soaking their bases in indole butyric acid (IBA) solution for 15 min prior to planting. Our results showed that rooting of small offshoots (less than 6 kg) can be significantly improved by exogenous application of IBA. Significant differences in rooting were clearly noted between the IBA treated offshoots and the untreated ones. This result constitutes a good evidence of the IBA effectiveness in rooting stimulation of date palm offshoots. Treatment with IBA solutions at 10 or 15 mg L^{-1} gave 96% of rooted offshoots while only 50% rooting was obtained with the untreated offshoots. Even small offshoots of less than 2 kg weight gave more than 90% rooting, if previously treated with 10 or 15 mg L^{-1} IBA solutions.

In addition, offshoots treated with 10 or 15 mg L^{-1} IBA solutions, could produce after 8 months, an average of 12 good roots per rooted offshoots, while the untreated and rooted offshoots tended to produce fewer roots that is around 4 roots per offshoot. These results are similar to those obtained by Qaddoury and Amssa (2004) on offshoots of the 'Mejhoul Moroccan' cultivar. The present encouraging results may be explained by the fact that exogenous application of IBA could have induced changes in endogenous enzymes peroxidase and IAA oxdase, allowing the establishment of a suitable cytokinin/auxin hormonal balance favorable to root formation (Qaddoury Amssa, 2004).

The high rooting percentage obtained in IBA treated offshoots was accompanied by a good offshoot vegetative growth. After 8 months, offshoots treated with 10 or 15 mg L^{-1} IBA solutions produced an average of 4 new leaves and their central leaf elongated by an average of 9 cm. Such results suggest that the high rooting percentage

obtained in the IBA treated offshoots would have been facilitated also by a good vegetative growth that has ensured a better photosynthetic activity that insured the necessary carbohydrates to sustain a good root induction and development.

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Tables

Table 1. Percentage of rooted offshoots and number of roots per offshoot as affected by IBA concentration. Values of the same column followed by different letters are significantly different (LSD at 5%).

IBA dose (mg L^{-1})	Rooting (%)	Number of roots per rooted offshoot
0	35.3a	3.75a
2	54.9a	7.25b
5	85.8b	9.25c
10	96.0c	10.88c
15	96.2c	14.11c

Figures

Fig. 1. Adult ‘Najda’ date palm vitroplant producing many offshoots in the field.

Fig. 2. ‘Najda’ date palm offshoots of the 4 weight classes made in the experiment.

Fig. 3. Offshoots under the experiment in the shade house.

Fig. 4. Rooted ‘Najda’ date palm offshoots derived from adult vitroplants, 6 months after the beginning of the experiment.

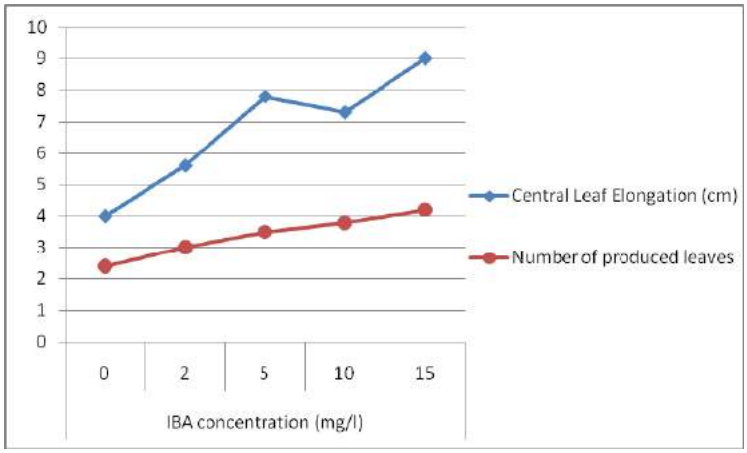

Fig. 5. Number of produced leaves and central leaf elongation according to IBA concentration.

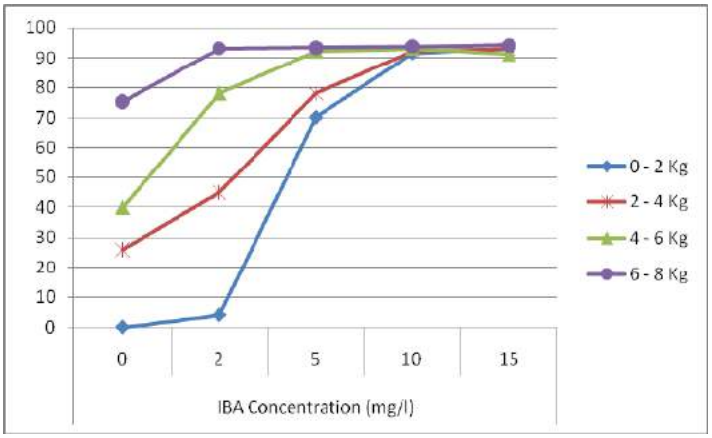

Fig. 6. Rooting percentage as affected by offshoot weight class and IBA concentration.

Determination of Performance Factors of Drip Irrigation for Date Palm (the Oasis of Gafsa: Southern Tunisia)

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Keywords: hydraulic conductivity, efficiency, uniformity coefficient, wets front, roots

Abstract

The interest of this study is the evaluation of a drip irrigation of date palm in an oasis region and specifically Aguila in the Governorate of Gafsa (south Tunisia), through some performance indicators such as the efficiency and uniformity of application. The first part of this work has been devoted to the determination of different soil hydrodynamic characteristics namely size, bulk density, infiltration of law and the hydraulic conductivity at saturation. Also work on the root distribution was conducted. Three profiles were sampled, one is 0.5 m from the palm trunk, the second to a distance of 1 m from the trunk of the palm and the third at a distance of 2 m. In a second step, an evaluation of irrigation has been conducted (under drip, drip of 15 cm and 30 cm from dripper). Three irrigation durations were tested for the same tree with a flow rate of 30 L/h (the rate adopted by the farmer). It is 2, 3 and 5 hours. The measures focused on monitoring the water content before irrigation, immediately after irrigation, after 24 and 48 hours after the end of irrigation for the three periods. The analysis of root distribution as a function of depth (0 to 120 cm) indicates that the majority of roots are located in the portion of soil between 40 and 120 cm. A follow-up range wetting the soil surface HR for the three periods of irrigation has been done. This radius is: -42 cm for irrigation of 2 hours duration, 48 cm for the irrigation of 3 hours duration, 60 cm for irrigation duration of 5 hours. The uniformity coefficient on moisture in the root zone was 99, 76 and 87% respectively for the duration of irrigation 5, 3 and 2 hours.

Détermination des Facteurs de Performance d'une Irrigation Goutte à Goutte pour le Palmier Dattier (l'Oasis de Gafsa: le Sud de la Tunisie)

Mots-clés: conductivité hydraulique, efficience, coefficient d'uniformité, front d'humectation, racines

Resumé

L'Intérêt de cette étude consiste en l'évaluation d'une irrigation goutte à goutte, dans une région oasienne et précisément dans le Gouvernorat de Gafsa, à travers certains indicateurs de performance telles que l'efficience et l'uniformité d'application.

La première partie de ce travail a été consacrée à la détermination de différentes caractéristiques hydrodynamiques du sol à savoir la granulométrie, la densité apparente, loi d'infiltration et la conductivité hydraulique à la saturation. Aussi un travail relatif à la répartition racinaire a été mené. Trois profils ont été prélevés l'un est de 0,5 m du tronc de palmier, le deuxième à une distance égale à 1 m par rapport au tronc du palmier et le troisième à une distance égale 2 m.

Dans une deuxième étape, une évaluation de l'irrigation a été menée (sous goutteur, 15 cm du goutteur et 30 cm du goutteur). trois durées d'irrigation ont été

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testées pour le même palmier avec un débit de 30 L/h (c'est le débit adopté par l'agriculteur) . Il s'agit de 2, 3 et 5 heures.

Les mesures ont porté sur un suivi de la teneur eau avant irrigation, juste après irrigation, après 24 et 48 heures après la fin de l'irrigation pour les trois durées. L'analyse de répartition des racines en fonction de la profondeur (0 à 120 cm) indique que la majorité des racines est localisée dans la tranche du sol comprise entre 40 et 120 cm.

Un suivi du rayon d'humectation à la surface du sol R_H pour les trois durées d'irrigation a été fait. Ce rayon est de:
- 42 cm pour l'irrigation de durée 2 heures.
- 48 cm pour l'irrigation de durée 3 heures.
- 60 cm pour l'irrigation de durée 5 heures.

On a constaté que les pertes en eau en deçà de la zone racinaire sont négligeables pour les trois durées d'irrigation et ne dépassent pas 10% du volume d'eau apporté. Le coefficient d'uniformité relatif aux teneurs en eau au sein de la zone racinaire a été de 99, 76 et 87% respectivement pour les durées d'irrigation 5, 3 et 2 heures.

INTRODUCTION

Les avantages du système de l'irrigation goutte à goutte, sont à l'origine de son extension rapide dans le monde: Les superficies équipées sont l'ordre $3{,}0.10^6$ hectares. En Tunisie, plus de 85% des ressources mobilisables sont déjà mises en exploitation dont plus de 80% destinées à l'irrigation. Parmi les meures préconisées à ce sujet, il y a l'extension des techniques d'irrigation localisée, dont particulièrement le système goutte à goutte. La superficie totale équipée dépasse 38000 hectares en 1999 grâce aux incitations de l'état (des subventions de 40 à 60% des coûts des projets (Daghari, 1983)). L'irrigation goutte à goutte est une technique qui permet d'augmenter les rendements et de réduire les pertes en eau c'est pour quoi il y a recours à ce système surtout dans les oasis en particulier les oasis de Gafsa.

Gafsa est une zone pré saharienne, souffrant depuis quelques années d'une sécheresse très lourde. Le climat est du type méditerranéen continental (hiver froid et été chaud). Les pluies sont faibles et irrégulières (120-220 mm/an), généralement orageuses à l'automne suivies par des longues périodes de sécheresse. La demande en évaporation est importante, elle est de 6 à 8 mm/j (CRDA de Gafsa, 2000).

Ce travail vise l'évaluation de cette technique d'irrigation dans une parcelle expérimentale retenue appartient à la région d'El Aguila dans la délégation de Gafsa sud.

Le travail est organisé comme suit:
- La caractérisation physique et hydrodynamique du site d'expérimentation ainsi que la description de la méthodologie employée.
- La présentation et la discussion des différents résultats.

MATERIEL ET METHODES

Les expériences ont été menées au sein d'une exploitation privée de onze hectares, située dans la zone "Aguila" de gouvernorat de Gafsa (sud tunisien). Le palmier dattier à proximité duquel, nous avons mené nos essais est de cinq ans d'age. L'irrigation adoptée par l'agriculteur est la goutte à goutte dont la durée est 5 heures; Le débit utilisé est de 30 L/h. Deux autres durées ont été testées à savoir 2 et 3 heures. Le goutteur est situé à 50 cm du tronc du palmier. A l'aide d'une tarière, on a fait des prélèvements des échantillons de sol aux profondeurs 20, 40, 60, 80, 100 et 120 cm; et ce-ci: sous goutteur, à 15 cm du goutteur et à 30 cm du goutteur pour les trois durées d'irrigation.

Le Profil Racinaire

Etant donnée que l'extraction racinaire constitue le principal facteur d'épuisement de l'eau dans le sol, on a fait des prélèvements d'échantillons (sol plus racines) à l'aide d'une tarière à différents niveaux allant de 20 à 120 cm avec un pas de 20 cm dans trois

profils différents. Ces prélèvements ont été faits à 0.5, 1 et 2 m par rapport au tronc du palmier.

RESULTATS ET DISCUSSIONS

L'objectif des essais expérimentaux réalisés in situ, dans la région d'El Aguila dans le gouvernorat de Gafsa est l'analyse de la répartition de l'eau au cours et entre deux irrigations successives, ainsi qu'une évaluation de l'irrigation.

Caractérisation Physique du Sol

Elle a porté sur l'analyse granulométrique, la détermination de la densité apparente et l'établissement de la loi d'infiltration.

Des échantillons de sol ont été prélevés aux profondeurs 20, 40, 60, 80, 100 et 120 cm. Les résultats d'analyse granulométrique obtenus montrent que le sol est de type sablo- limoneux. La densité apparente sèche du sol a été déterminée. Elle varie entre 1.6 et 1.8 avec une moyenne de 1.7 (Fig. 1).

Infiltration

La conductivité et la sorptivité sont estimés de façon empirique à partir des mesures de l'infiltration sur terrain. L'étude de l'infiltration permet de déterminer la conductivité à la saturation K_s ainsi que la sorptivité (Figs. 2 et 3): K_s=9, 2 cm/h et s=10, 44.

Analyse de la Répartition Racinaire

Il est difficile de faire l'interprétation correcte de la répartition des racines. Pour le premier palmier, on note la présence du Gazon tout autour. Il est impossible de distinguer entre les racines du palmier et ceux du Gazon (Fig. 4 et 5). Pour le deuxième palmier sans gazon, la répartition des racines devient plus claire. On a constaté que la majorité des racines est localisée dans la tranche du sol comprise entre 20-120 cm.

Analyse de la Répartition de l'Eau

1. Evolution du Rayon d'Humectation à la Surface du Sol. Le rayon R_H ne dépasse pas 42, 48 et 62 cm respectivement pour les durées 2, 3 et 5 heures d'irrigation (Fig. 6).

2. Evolution de la Teneur en Eau.

Evolution de la Teneur en Eau Initiale. La teneur en eau moyenne avant l'irrigation est de l'ordre de 15%. Cette valeur est la même pour les trois durées d'irrigation testées.

Cas de l'Irrigation de Durée Deux Heures. Les résultats montrent que la valeur moyenne de la teneur en eau juste à la fin de l'irrigation est d'environ 22%. Cette valeur diminue pour atteindre 18% après 24 heures. Après 48 heures, la teneur en eau chute à 15% (Fig. 7).

Cas de l'Irrigation de Durée Trois Heures. La teneur en eau moyenne initiale et à la fin de l'irrigation est respectivement de 15 et de 27%. Elle diminue par la suite pour atteindre 22% après 24 heures. On assiste à un retour à l'état initial 48 heures après la fin de l'irrigation où la teneur en eau moyenne devient 17%.

On remarque que la teneur en eau moyenne à la fin de l'irrigation est de 33%. Cette valeur diminue progressivement au cours de la phase de la redistribution de l'eau dans le sol pour arriver à 31% après 24 heures et 25% après 48 heures (Fig. 8).

ETUDE COMPARATIVE DE TROIS DUREES D'IRRIGATION

Pour comparer les trois durées d'irrigation, on a essayé d'évaluer les paramètres suivants:

- l'efficience.
- le coefficient d'uniformité.
- la teneur en eau moyenne.

Efficience de l'Irrigation

Etant donné que l'efficience de l'irrigation représente l'augmentation du stock

d'eau dans la zone racinaire rapportée au volume fourni, nous avons calculé les variations des stocks d'eau dans le volume du sol contrôlé.

Pour cela, on a divisé le volume du sol en petits domaines cylindriques de dimensions Δr_i et Δz_j, centré en M (ri, zj) où la teneur en eau est supposée constante.

Vu qu'il nous est difficile de déterminer avec exactitude la profondeur de la zone racinaire, puisque notre plante d'étude est un palmier, on a retenu une profondeur de 120 cm.

Coefficient d'Uniformité

Le coefficient d'uniformité Cu relatif à la teneur en eau au sein de la zone racinaire est de 94% avant irrigation. Le sol est relativement sec. Ce coefficient est passé à 78, 76 et 99% respectivement pour les durées 2, 3 et 5 heures.

En se référant au tableau n°1, à la fin de l'irrigation, le sol a été le plus uniformément humidifié dans le cas de l'irrigation de durée 5 heures (Fig. 9). Pour les trois durées, la teneur en eau à la capacité au champ (16%) a été largement dépassée même si la variation du stock d'eau ne témoigne pas d'une perte d'eau notable.

CONCLUSION GENERALE

Le présent travail a été consacré à l'évaluation de l'irrigation goutte a goutte dans un contexte aride dans la région de Gafsa du sud tunisien.

La parcelle expérimentale, plantée en palmeraies, fait partie des périmètres privés irrigués à partir d'un forage. L'unité d'arrosage est constituée d'un bassin de diamètre 7 m.

Le réseau de distribution est composé des rampes installées en parallèles dont les goutteurs sont espacés l'un par rapport à l'autre de 10 m.

Différentes durées d'irrigation ont été utilisées à savoir 2, 3 et 5 heures avec un débit de 30 L/h.

L'analyse de la répartition des racines montre que, latéralement, la majorité des racines est localisée dans un cylindre de rayon 30 cm autour de la racine principale. Perpendiculairement, on constate que la majorité des racines se trouve au niveau de la tranche du sol comprise entre 40 et 120 cm.

En ce qui concerne le rayon du front d'humectation (R_H), on a relevé que ce paramètre ne dépasse pas 50 cm.

On ne peut pas prendre l'efficience comme critère de comparaison; en effet, les lames d'eau perdues pour les trois durées d'irrigation, sont négligeables (10% de lame d'eau apportée pour t=2 h, 7% pour t=3 h et pour t=5 h).

L'analyse de l'uniformité au sein du volume du sol contrôlé témoigne d'une bonne uniformité de l'irrigation pour la durée d'irrigation de 5 heures qui est de 99% à la fin de l'irrigation même si la teneur en eau à la capacité au champ a été largement dépassée. Dans nos travaux futurs, il faut confronter plusieurs approche quant à:

- La mesure de flux d'évapotranspiration par la méthode de flux de sève car il est peut être anormal d'absever une vitesse de 23 mm/j.
- La partition entre l'évapotranspiration et le flux profond de drainage.
- La caractérisation de la zone d'activité racinaire.

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Tableaux

Tableau 1. Volumes d'eau pour les différentes durées d'irrigation.

Durée d'irrigation	2 heures	3 heures	5 heures
Volume apporté (L)	60	90	150
Volume initial (L)	114	114	114
Volume final (L)	168	200	253
Variation de stock (L)	54	86	140
Volume perdu (L)	6	4	10

Tableau 2. Teneurs en eau moyennes θ_{moy} et coefficients d'uniformité C_u pour les différentes durées d'irrigation.

Durée d'irrigation		Avant l'irrigation	A la fin de l'irrigation	24 h après la fin de l'irrigation	48 h après la fin de l'irrigation
2 h	θ_{moy}	15	22	18	15
	C_u	94	78	82	90
3 h	θ_{moy}	15	27	22	17
	C_u	94	76	97	98
5 h	θ_{moy}	15	33	31	25
	C_u	94	99	98	97

Figures

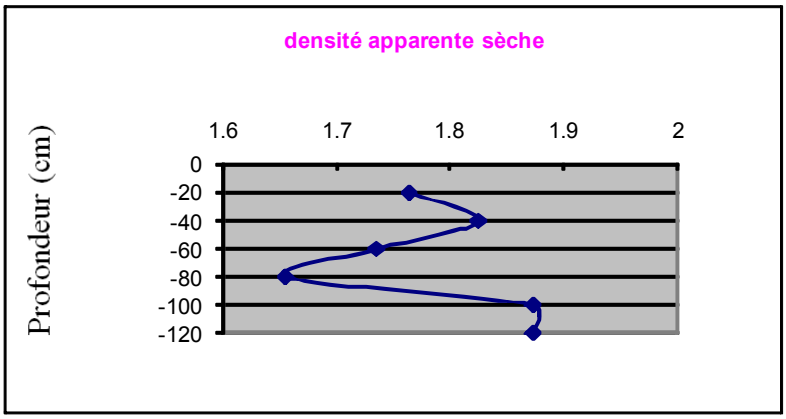

Fig. 1. Densité apparente sèche.

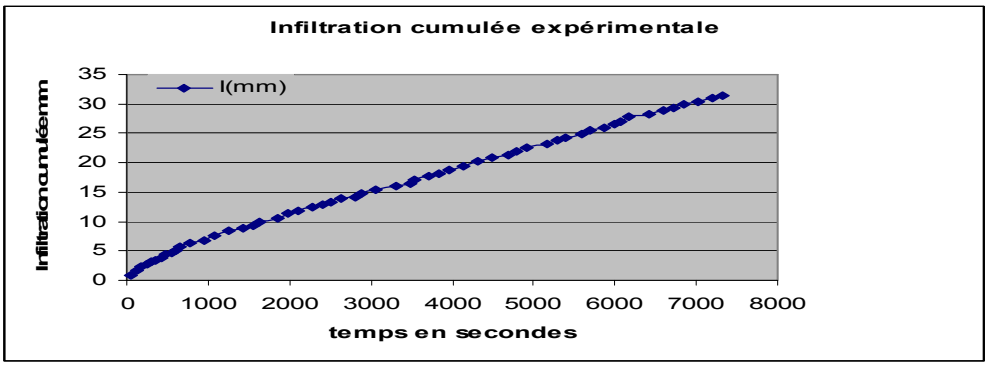

Fig. 2. Infiltration cumulée expérimentale.

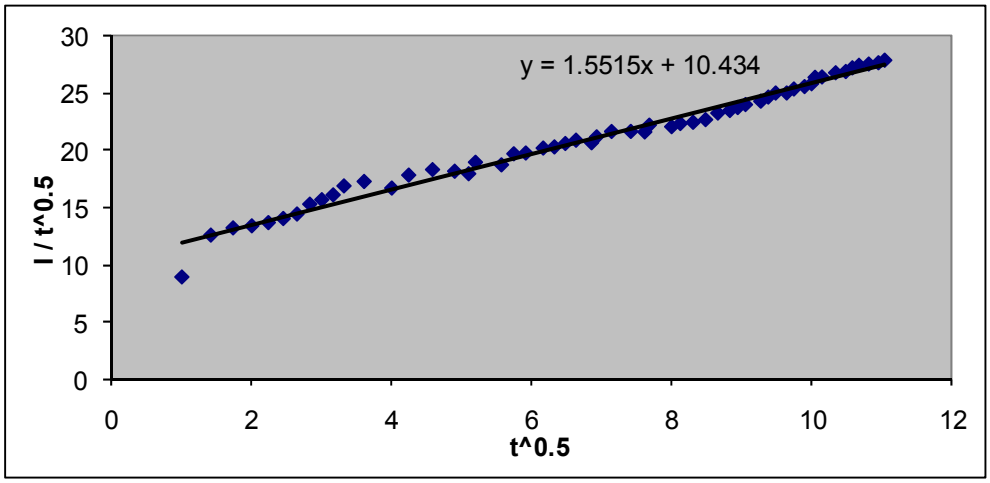

Fig. 3. Détermination de la conductivité hydraulique à la saturation.

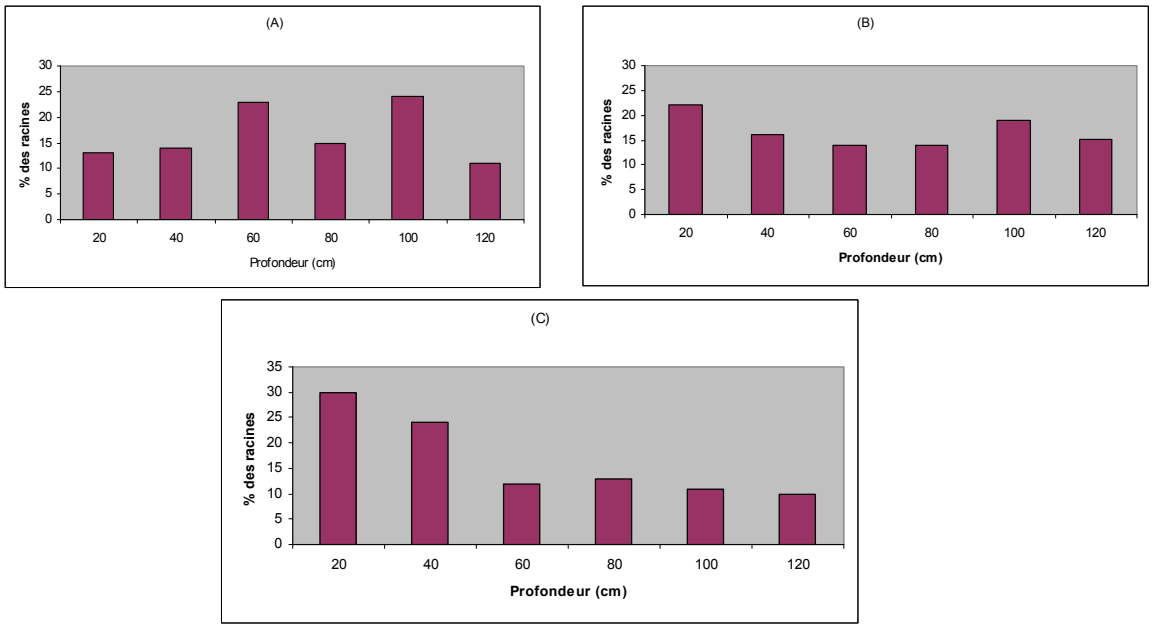

Fig. 4. Répartition verticale des racines de notre palmier d'étude (A): distance r=0,5 m; (B): distance r=1 m; (C): distance r=2 m par rapport au tronc du palmier.

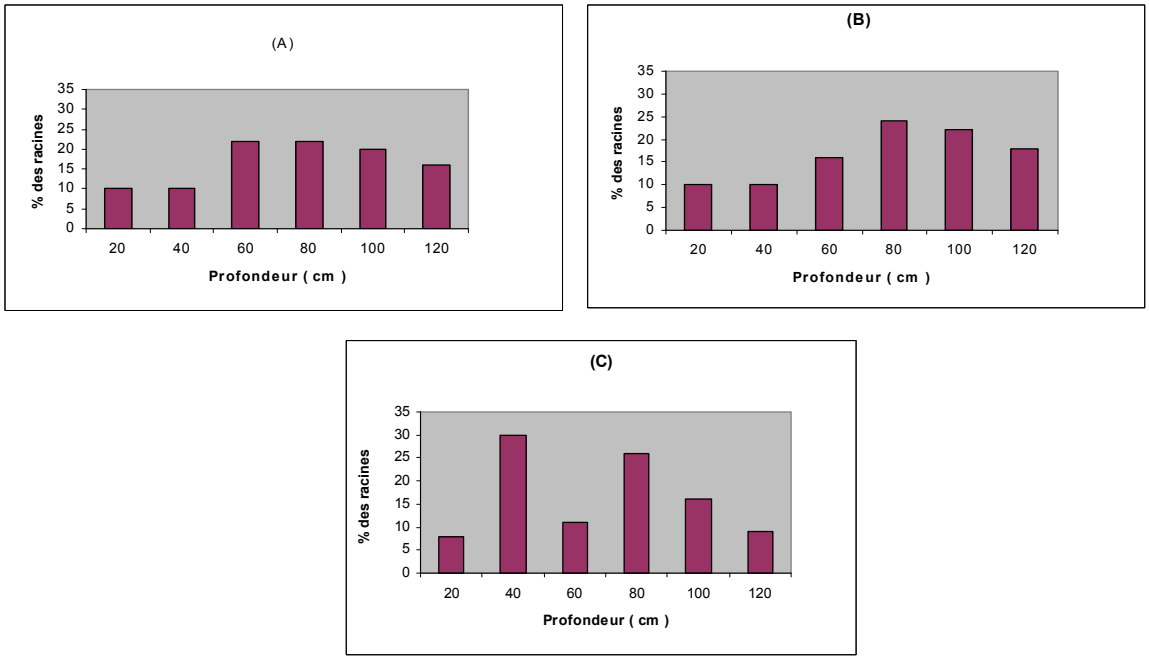

Fig. 5. Répartition verticale des racines d'un autre palmier (A): distance r=0,5 m; (B): distance r=1 m; (C): distance r=2 m par rapport au tronc du palmier.

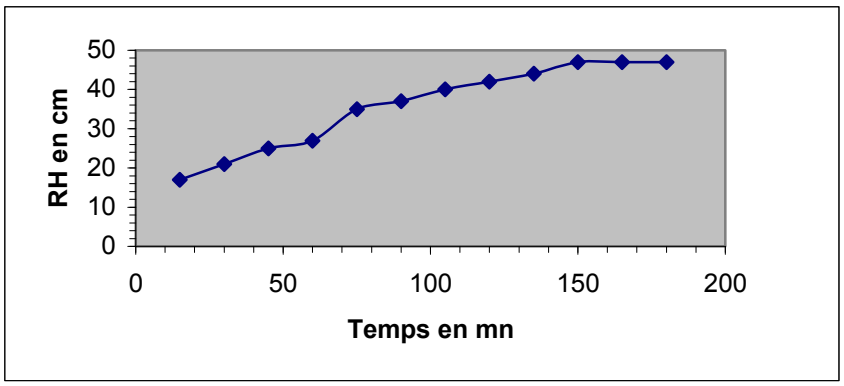

Fig. 6. Evolution du rayon humidifié R_H, à la surface du sol en fonction du temps (cas de l'irrigation de durée 3 heures).

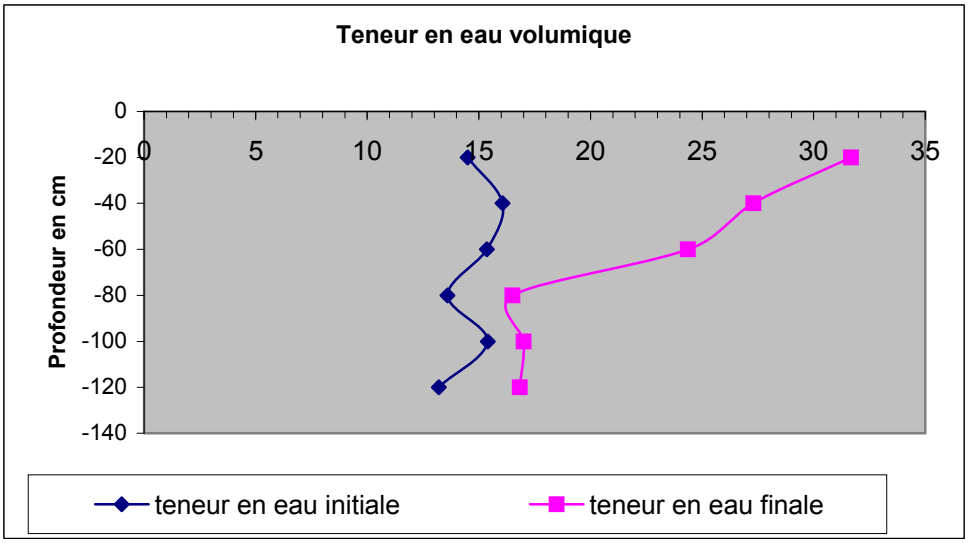

Fig. 7. Profils hydriques sous goutteur avant et à la fin de l'irrigation de durée 2 heures.

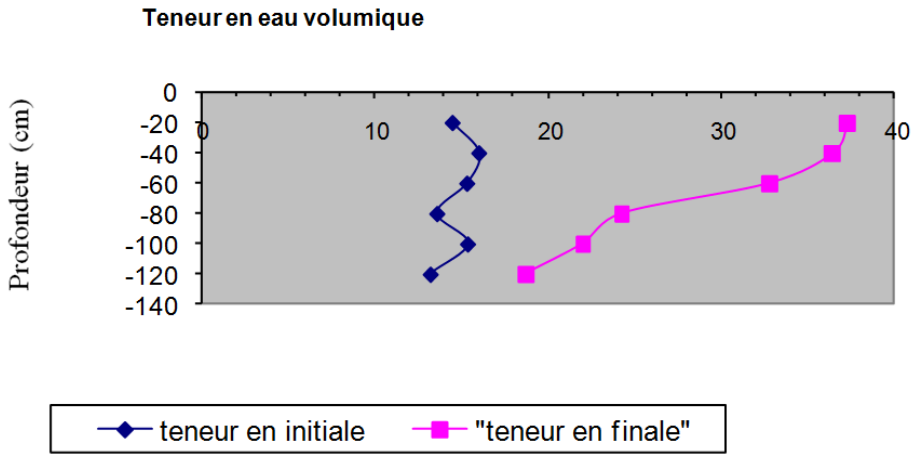

Fig. 8. Profils hydriques sous goutteur pour la durée d'irrigation 3 heures.

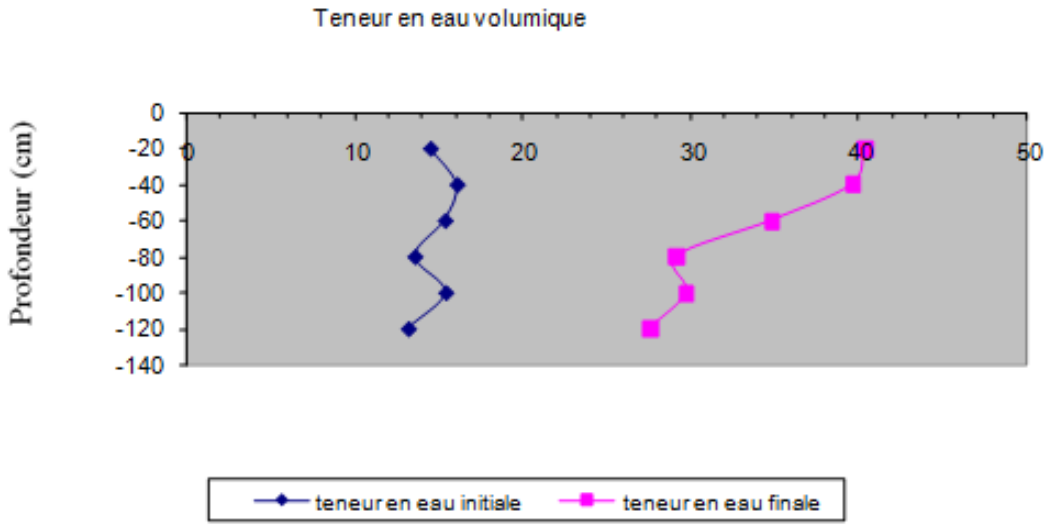

Fig. 9. Profils hydriques sous goutteur au temps initial et à la fin de l'irrigation pour l'irrigation de durée 5 heures.

Comparative Study of Mechanical and Hand Pollination on Certain Omani Date Palm Cultivars

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Keywords: mechanical pollination, Sultanate of Oman

Abstract

This study was carried out at the Wadi Quriyat Date Palm Research Station, and Tanuf farm, Bahla Governorate, Sultanate of Oman for comparing mechanical and hand pollination methods. The experiment was conducted on five female date palm cultivars i.e., 'Khalas Aldahra', 'Gibri', 'Hilali', 'Zabad' and 'Fard' for three seasons 2002, 2003,and 2004. Three other cultivars; 'Khalas Oman', 'Khandesi' and 'Khasab' were studied in 2003 and 2004. The results of two pollination methods, hand and mechanical, showed no significant differences in fruit set percentage for all cultivars under study at the Wadi Quriyat Date Palm Station and Tanuf farm. Moreover, the data indicated no statistical differences in the yield produced by either mechanical or hand pollination at both experimental sites.

In conclusion, since the quantity of pollen grains used is lower and less labor is required in mechanical pollination, it is a more economical method compared with hand pollination.

INTRODUCTION

Hand pollination cannot be done adequately and early enough, because of non-availability of specialized labor and the resultant higher cost. Moreover, the height of date palm trees makes the hand pollination difficult.

Mechanical pollination from ground level for four times with 1:10 (pollen:filler ratio) increased the total yield of 'Zahdi' (Hamood et al., 1986; Shabana et al., 1986).

Obviously, the frequencies of mechanical pollination and the choice of the suitable concentration of pollen:filler ratio are considered the most important factors in the date palm pollination.

The objective of this study is to compare mechanical pollination with hand pollination on fruit set and quality of some Omani date palm cultivars.

MATERIALS AND METHODS

The study was conducted during 2002, 2003 and 2004, to compare mechanical pollination with hand pollination. Two locations i.e., the Wadi Quriyat Date Palm Research Station and Tanuf farm were chosen to carry out this experiment.

Wadi Quriyat Date Palm Station

For the seasons 2002, 2003 and 2004 'Khalas Aldahra', 'Hilali', 'Gibri', 'Zabad' and 'Fard' were chosen. Moreover, in 2003 and 2004 other cultivars were chosen; 'Khalas Oman', 'Khanezi' and 'Khasab'. Ten uniform date palm trees were selected with a height of 2 to 2.5 m for each cultivar under study.

Tanuf Farm

Five uniform date palm trees of 'Khalas Aldahra' and 'Fard' of 22 years and height 7-8 m were selected.

The male spathes were cut out as soon as cracked and taken to a dry room at 28-32°C with good ventilation. The extractions of pollen grains were carried out by the traditional hand method. Then, the fresh pollen was mixed with flour as filler at ratio of 1

pollen:7 flour. This mixture was sprayed at five times frequencies at an interval of 4-5 days.

The mechanical pollination was done by using the manual pollinator machine manufactured by the Ministry of Agriculture and Fisheries, Sultanate of Oman.

The hand pollination treatment was done three times by inserting 4 to 5 male strands in the female spadix.

For storage, the surplus fresh pollen was placed in plastic container (250-300 g) with cover and kept in a refrigerator at 4-6°C. This stock can be used either in subsequent application, or in the next season.

The experiment was designed in a Randomized Complete Block using whole tree replication with ten fruit bunches per palm. All data were subjected to analysis of variance and means were compared by the least significant differences at the 5% level.

The parameters under study included: fruit set and yield. Percentage of fruit set was determined as described by Ream and Furr (1970). Yield was determined by the total weight of ten bunches for each replication and then total yield was calculated.

RESULTS AND DISCUSSION

The criteria used in selecting the suitable pollinator are, ease to use, light weight and its efficiency to place the pollen grains to the female spathes. The length of the palm tree might be also considered as another important factor. That might increase the time and the effort spent by the laborers in conducting the pollination.

Table 1 shows that the time needed to pollinate a palm 1.5 to 2.5 m tall (8 years) mechanically is 1 to 1.5 min at the Wadi Quriyat Station. While this time was doubled with 6 to 7 m tall palm trees at Taunt farm. However, hand pollination approximately need 10-15 min to pollinate a tall palm while it took 5-8 min for a short palm. Moreover, a very little amount of pollen grains 2-3 g/palms were used in the mechanical method compared with hand pollination (7-8 g).

Fruit Set

No significant differences in fruit set were observed for all the cultivars under study both at the Wadi Quriyat Date Palm Station and Tanuf Farm (Tables 2 and 3 and Figs. 1 and 2). The fruit set (%) of the subsequent seasons i.e., 2003 and 2004 was in conformity with results of the earlier study. The studied cultivars at the two locations also revealed no significant differences in the fruit set by either methods of pollination (hand and mechanical). These results agree with the findings of Brown and Perkins (1969, 1970) and Nixon (1978) who adopted the mechanical pollination in USA. Moreover, Shabana et al. (1986) recommended the mechanical method of pollination under Iraq conditions.

Yield

The data presented in Table 4 and Figure 3 showed that the yield of the ‘Fard’ and ‘K. Aldahra’ depicted no significant differences between the two methods of pollination under Tanuf farm conditions. Moreover, the date palm cultivars under study at Wadi Quriyat Date Palm Station indicated no differences in the yield produced by either mechanical or hand pollination (Table 5 and Fig. 4). These results are in harmony with the finding of Hamood et al. (1986) and Shabana et al. (1986) who stated that mechanical pollination from ground level repeated four times improved the yield of ‘Zahdi’.

In conclusion, these results indicated that the mechanical pollination repeated at a 4-5 times interval have improved fruit set and the yield, though not significantly. However, if we compare the lower amount of pollen grain and the lesser time required in mechanical pollination, it is comparatively a more economical method of pollination both for tall and short palms.

In view of encouraging results of the concluded experiment it is intended to lay out the experiment in the farmer’s field with the objective of achieving reproducible results.

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Tables

Table 1. Palm height, pollen grain quantity, required time, and frequencies by pollination methods and number of pollinated palm.

Pollination method	Palm height (m)	Pollen grain (g/palm)	Required time for pollination (min./palm)	Pollination frequencies	No of palms/ hour
Hand	6-7	7-8	10-15	2-3	6-5
Mechanical	6-7	2-3	2-3	4-5	20-30
Hand	1.5-2.5	7-8	5-10	2-3	6-12
Mechanical	1.5-2.5	2-3	1-1.5	4-5	45-60

Table 2. Effect of pollination methods on the fruit set (%) of some date palm cultivars at Tanuf farm (means for three seasons 2002, 2003 and 2004).

Method of pollination	Cultivars	
	Fard	Khalas Aldahra
Mechanical	77	59
Hand	76	58
LSD	NS	NS

NS: Not significant

Table 3. Effect of pollination methods on the fruit set (%) on some date palm cultivars at Wadi Quriyat Date Palm Station.

Method of pollination	Cultivars						
	K.Aldahra*	Gibri*	Hilali*	Zabad*	Kanezi**	Khasab**	K. Oman**
Mechanical	60	75	85	62	62	75	74
Hand	59	74	86	63	62	74	73
LSD	NS	NS	NS	NS	NS	NS	NS

* Mean of three seasons 2002, 2003 and 2004.
** Mean of two seasons 2003 and 2004.
NS: Not significant.

Table 4. Effect of pollination methods on the yield (kg) of some date palm cultivars at Tanuf farm (means for three seasons 2002, 2003 and 2004).

Method of pollination	Cultivars	
	Fard	K. Aldahra
Mechanical	52	44
Hand	50	41
LSD	NS	NS

NS: Not significant

Table 5. Effect of pollination methods on the yield (kg) of some date palm cultivars at the Wadi Quriyat Date Palm Research Station.

Method of pollination	Cultivars						
	K. Aldahra*	Gibri*	Hilali*	Zabad*	Kanezi**	Khasab**	K. Oman**
Mechanical	45	45	53	41	34	35	35
Hand	39	42	50	37	35	38	34
LSD	NS	NS	NS	NS	NS	NS	NS

* Mean of three seasons 2002, 2003 and 2004.
** Mean of two seasons 2003 and 2004.
NS: not significant.

Figures

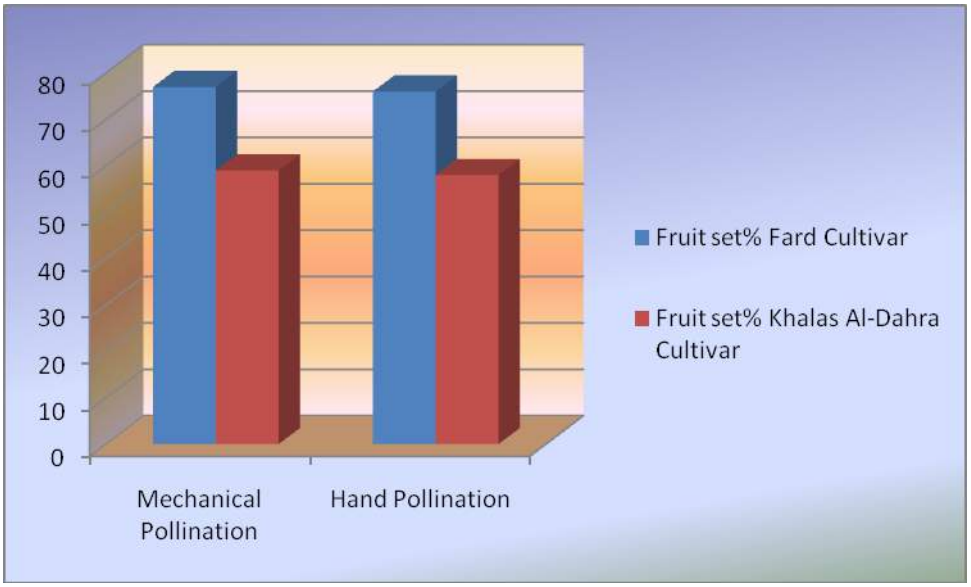

Fig. 1. The effect of pollination methods on the fruit set % for two dates palm cultivars at Tanuf farm.

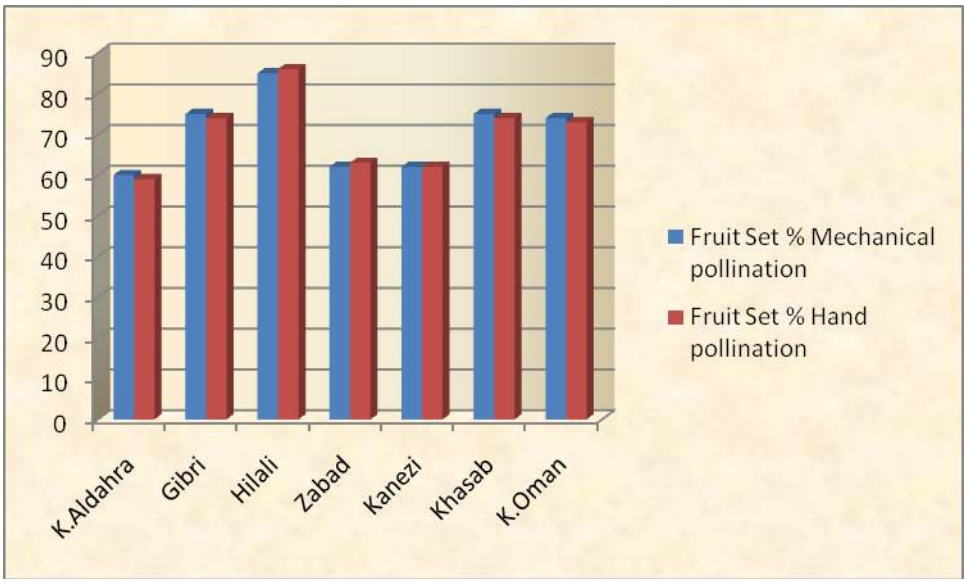

Fig. 2. The effect of pollination methods on fruit set % on some date palm cultivars at the Wadi Quriyat Date Palm Station.

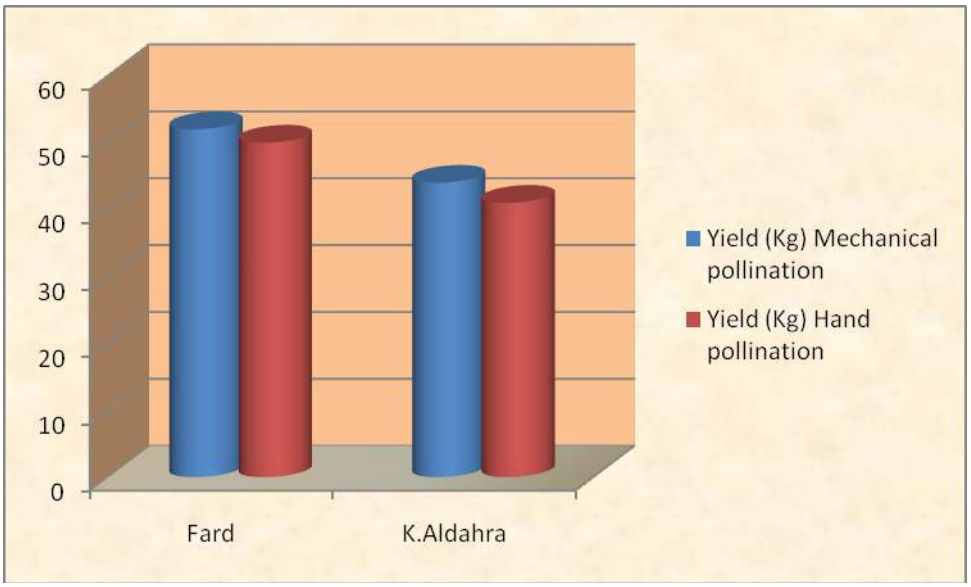

Fig. 3. The effect of pollination methods on the yield of two date palm cultivars at Tanuf farm.

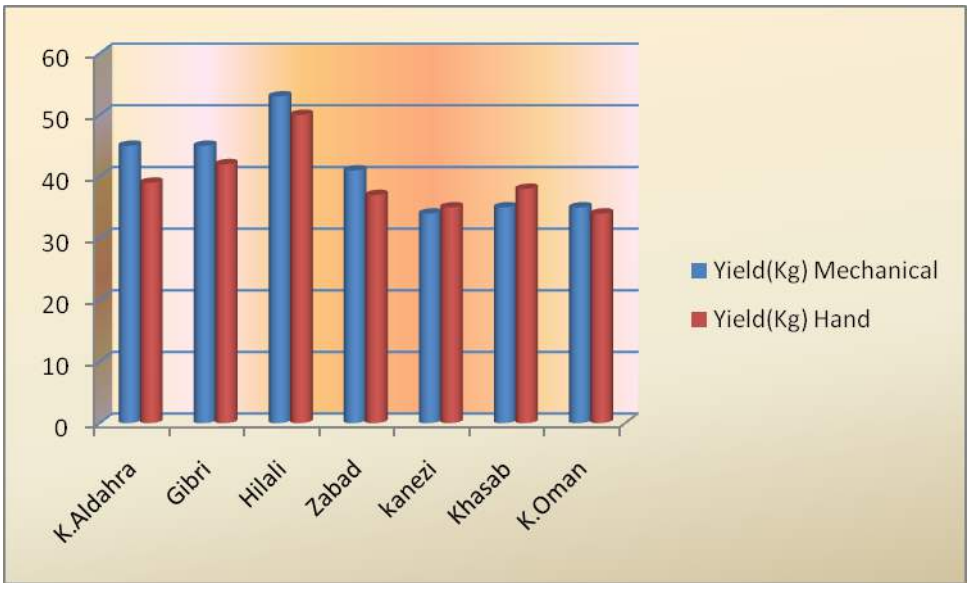

Fig. 4. The effect of pollination methods on the yield of some Omani date palm cultivars at the Wadi Quriyat Date Palm Station.

The Effect of Thinning on Fruit Drop of Date Palm 'Khanezi'

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Keywords: thinning, date Fruit drop, Sultanate of Oman

Abstract

This study was conducted at the Wadi Quriyat Date Palm Research Station, Bahla Governorate, Sultanate of Oman in 2002, 2003, and 2004 to investigate the pattern of date palm 'Khanezi' fruit drop and the effect of thinning treatments on reducing fruit drop. In addition the experiment consisted of three treatments; thinning ¼ of total number of bunches, thinning ¼ of total strands per bunch, and control (no thinning). The experiment was designed as Complete Randomized Block Design (CRBD) and each treatment had three replicates.

The results showed that there were two waves of heavy fruit drop; the first one onset 60 days after fruit set while the second wave onset after 90 days after fruit set. The strand thinning treatment decreased fruit drop in the second wave and gave a higher yield in comparison with bunch thinning and control treatments.

INTRODUCTION

It is necessary to explain the Omani terms; khalal and bisir which will be mentioned later in this study. Khalal denotes the green stage of the date fruit, while bisir is the stage of turning the fruit color from green to red. Observations on a number of date palm groves located at similar environmental conditions for different successive seasons indicated that the date palm cultivar 'Khanezi' suffers from fruit drop at certain stages of fruit development. Fruit set and fruit drop vary even within the same region. Reuveni (1969) established the main facts for fruit drop. First, the rate of natural fruit drop varies with cultivar and garden. Second, the rate of drop is more or less fixed for each cultivar in each garden. Third, the pattern of fruit drop through the season is specific for each cultivar. Mawloud et al. (1997) recorded three waves of fruit drop with cultivar 'Zabad' at the same grove; Wadi Quriyat Date Palm Station. The first started 25 to 30 days after fruit set at a rate of 16 to 20%. The second was 60 days and the third 70 days after fruit set. The high percentage of 'Khanezi' fruit drops at khalal and/or bisir could be attributed to nutritional status, agricultural practices and/or environmental conditions. Fruit and fruit bunch thinning experiments should be designed according to the general conditions of date orchard as affected by irrigation, fertilization and age of the trees (Mawloud, 2001). The beneficial effect of fruit and/or fruit bunch thinning on the quality of fruit is well known. Both techniques of thinning help to improve size, quality and prevent delayed ripening. Furthermore, it reduces the compactness of the fruit bunches, overcome the problem of the alternate bearing and reduce fruit drop. In a recent study, Um Alzein (1998) found that thinning bunches or number of fruits improved quality and increased fruit size by 5-10%.

The objectives of this study were: 1. to study the pattern fruit drop of cultivar 'Khanezi'; 2. to study the effect of thinning treatments on reducing the percentage of fruit drop.

MATERIALS AND METHODS

The experiment was carried out in 1999, 2000, 2001 and 2002 at the Wadi Quriyat Date Palm Station. Nine uniform date palm trees of cultivar 'Khanezi' were selected. Treatments were replicated three times using whole tree replication in Completely

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Randomized Block experiment. The aim was to study the effect of thinning treatments on fruit drop. The experiment was a completely randomized block design consisting of two treatments, thinning ¼ of total number of bunches and thinning ¼ of total strands per bunch with a control (no thinning) and each treatment had three replicates.

Thinning was carried out after two weeks from pollination in the 1st season (1999), while in the rest of the seasons thinning was applied only at the time of pollination. Since the date of applications of the first seasons was different from subsequent dates in 2000, 2001 and 2002, it was dropped from the analysis. The standard error was used to compare the means of the treatments.

The following characteristics were studied:

1. Fruit drop percentage: the fruit drop was counted once every 15 days. Cumulative fruit drop expressed as percentage of total dropped period from green stage; khalal, bisir, rutab and tamar stages.
2. Fruit set percentage: five strands were taken from ten bunches randomly. The fruit set percentage was calculated.
3. Yield: the weight of bunches at fully rutab stage was used to determine the yield by kg.
4. Fruit weight and dimensions: 20 fruit from each replicate of all treatments were taken to determine the following characteristics: fruit weight was determined by weighing the average of fruit sample (g). Fruit dimensions length and diameter of individual fruit were measured (cm) by vernier caliper. Average weight (g) of the seeds was also recorded.
5. Total soluble solids: total soluble solids of the fruit at tamar stage were determined by a refractometer (Model Atago).

RESULTS

Fruit Drop

The effects of thinning treatments on fruit drop of date palm cultivar 'Khanezi' are presented in Table 1. The course of fruit drop was characterized by two distinctive waves. The first one is at the end of the khalal stage (60 days after pollination), and the second wave is at the end of the bisir stage (90 days after pollination). Generally fruit drop of 'Khanezi' varied with the stages of fruit development from low at the beginning of the khalal stage to high with two peaks at the end of the khalal as well as at the end of the bisir stage. Moreover, the fruit drop wave was relatively low at the end of the tamar stage (Fig. 2).

Fruit Set

No significant differences in fruit set were found between thinning treatments (Table 2), which is in agreement with Nixon (1935) who showed that the fruit set percentage did not vary when different thinning treatments were employed.

Yield

Thinning treatments increased the yield of date palm 'Khanezi' significantly (Table 2 and Fig. 2). These results agree with Nixon and Carpenter (1978). However, the yield showed no significant differences between strand and bunch thinning techniques. (Al-Bekr, 1972) indicated that shortening inflorescences and cutting strands from the center of the cluster to make them more open for air movement through them has given the best results.

Fruit Weight

Data presented in Table 2 show that strand thinning significantly increased fruit weight and that bunch thinning gave heavier fruit.

Fruit Dimensions

Fruit length and diameter increased by either strand or bunch thinning, a finding

similar to that of Um-Alzein (1998) who found that thinning increased date fruit size (Table 3).

Seed Weight

Thinning decreased seed weight, indicating that the flesh of date fruit has increased (Table 3).

Total Soluble Solids

the data of soluble solids presented in Table 3 show no significant differences among the treatments.

DISCUSSION

The trend of fruit drop was found to be similar in the different thinning treatments. However, strand and/or bunch thinning decreased the percentage of fruit drop slightly. The results agree with Reuveni (1969) who indicated that the degree of thinning does not influence the percentage of natural drop. Furthermore, he concluded that the degree of fruit set and natural drop are fixed for each cultivar in each garden. These findings support the present results. However, there is still a need for further investigation to find out the reasons for the different behavior pattern displayed by the 'Khanezi' date palm cultivar at different stages of fruit development.

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Tables

Table 1. Effect of thinning treatments on fruit drop of date palm cultivar 'Khanezi'.

Treatments	Fruit drop percentage (%) at different fruit stages								
	Khalal stage		Bisr stage			Rutab and Tamar stages			
	20th May	4th June	20th June	4th July	18th July	2nd Aug.	16th Aug.	28th Aug.	2nd Sept.
Strand thinning	10.8	18.7	6.0	25.1	2.0	4.3	3.1	3.5	1.0
Bunch thinning	10.3	23.1	6.9	34.3	4.4	6.2	3.8	3.5	1.9
Control	11.0	29.5	10.5	36.4	6.9	8.1	10.4	4.5	1.8

Table 2. Effect of thinning treatments on fruit set and the yield of ‘Khanezi’ date palm.

Treatments	Fruit set (%)	Yield (kg)
Strand thinning	68.0	68.3
Bunch thinning	68.6	60.3
Control	67.6	55.6
SE	±0.882	±6.245**

Table 3. Effect of thinning treatments on some physical and chemical characteristics.

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Seed weight (g)	TSS (%)
Strand thinning	8.9	3.7	2.1	0.56	67.0
Bunch thinning	8.6	3.5	2.0	0.66	66.3
Control	8.2	3.3	1.9	0.73	66.3
SE	±0.578**	±0.321**	±0.203**	±0.145NS	±0.577NS

Figures

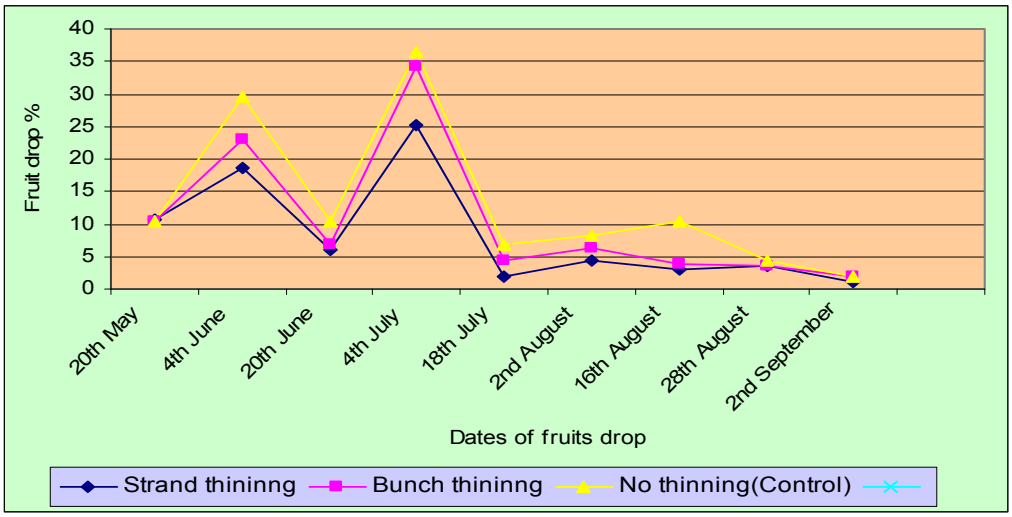

Fig. 1. Effect of thinning treatments on fruit drop of date palm cultivar ‘Khanezi’.

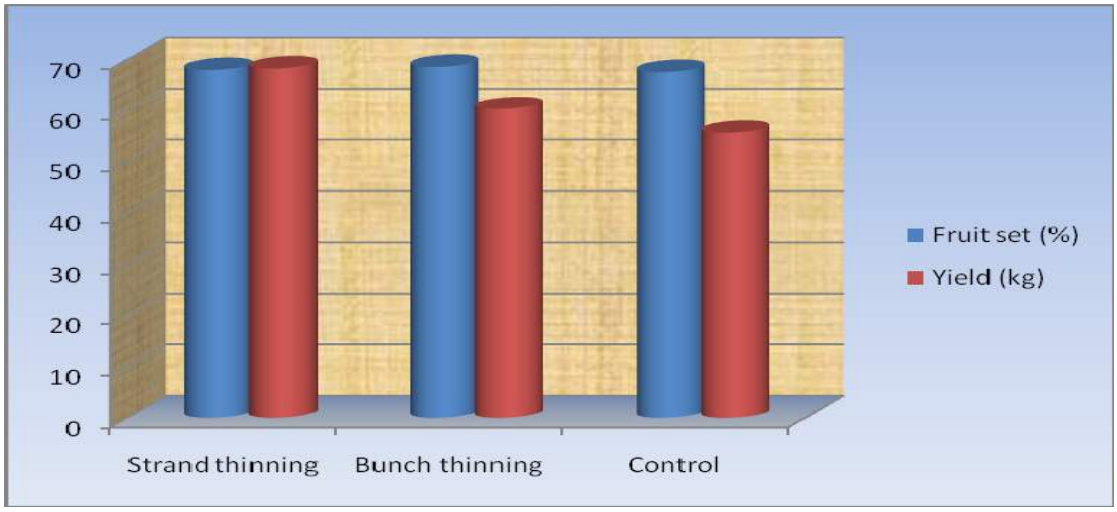

Fig. 2. The effect of thinning treatments on fruit set and the yield of date palm cultivar 'Khanezi'.

Interactive Effects of Temperature and 1-Methylcyclopropene on 'Barhee' Date Fruit Quality Picked at Khalal Stage

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Keywords: *Phoenix dactylifera*, 1-MCP, postharvest, storage, quality

Abstract

The fruit of date palm (*Phoenix dactylifera* L.) 'Barhee' is mainly harvested at khalal stage when it is physiologically mature, crisp with over 50% moisture content and it is very perishable. Fruit softening, appearing rutab spots and skin wrinkling are the main problems restrict that marketing and storage of khalal dates. In this study, postharvest application of 1.2 µl/L 1-methylcyclopropene in modulating fruit ripening and quality attributes under three storage temperatures (5, 15 and 30°C) was studied. Fruits were analyzed in four days intervals up to 28 days of storage. Results showed that weight loss, skin wrinkling, rutab spots, softening and surface color were affected significantly by storage temperature. However, the effect of treatment with 1-MCP was negligible on most studied quality attributes but for fruits stored under 5°C, 1-MCP considerably modulated weight loss, flesh softening, rutab spots area and darkening.

INTRODUCTION

Phoenix dactylifera commonly known as the date palm is widely cultivated in the arid region of the Middle East and north Africa. Date is a berry fruit of which the development is divided into five stages namely, hababook, kimri, khalal, rutab and tamar. Most date cultivars are harvested at tamar stage when the fruit is fully matured. 'Barhee' is one of the most popular date cultivars whose fruit is mainly harvested and consumed at khalal stage when they are physiologically mature, hard and crisp and bright yellow in color (Barreveld, 1993). At this stage fruit has over 50% moisture content and is very perishable. The main limitations in its transport and marketing is occurrence of fruit softening which is accompanied by appearing of rutab spots and surface wrinkling (Mortazavi et al., 2007). It seems that ethylene is involved in quick physiologically changes developing after harvesting khalal date fruit. 1-methylcyclopropene (1-MCP) has been shown to reduce ethylene action and to delay softening in many fruits (Watkins, 2006). Also temperature reduction has been seen to control postharvest disorders in different fruit such as apricots (De Martino et al., 2002), apples (Saltveit, 1984) and kiwifruit (Mencarelli et al., 1996).

However, the effects of 1-MCP on postharvest physiological changes of date fruit have not been investigated, therefore, the aim of this study was to investigate the effects of application of 1-MCP on 'Barhee' date fruit quality picked at khalal stage and stored at different storage temperatures.

MATERIALS AND METHODS

Date fruit 'Barhee' was harvested at khalal stage from a commercial orchard in Ahvaz, Khuzestan province, Iran. Fruit at commercial maturity (yellow skin color, firmness 55.5±1 N and TSS 43±0.76%) of uniform size, free from any rutab spots or blemishes were chosen for the experiment. The fruit were precooled immediately, then washed with sodium hypochlorite solution 0.5% for 2 min, rinsed with tap water and dried prior to treatment. Fruits were divided into two lots, separately kept in hermetically sealed plastic boxes (70×70×50 cm) and exposed to ambient air (control) and 1.2 µl L^{-1}

1-MCP for 24 h at 20°C. Following 1-MCP treatment fruits were stored at 5, 15 and 30°C and 80-90% RH up to 28 days. Visual examinations and other quality attributes were evaluated initially and periodically at the four day intervals. The fruit subjected to all treatments were weighed before and after storage and data expressed as percentage of weight loss. Fruit firmness was measured by a firmness tester (Lutron, FG-5020). Titratable acidity was calculated as percentage of malic acid by titrating 10 g/100 ml of the date extract with a solution of 0.01 N NaOH till pH 8.1. The pH was measured by a Metrohm pH meter. The level of sugars was measured as °Brix by a digital Atago refractometer. Outer surface color was determined colorimetric and reported as lightness, hue angle and Chroma and flesh darkening was followed by fruit extract absorbance at 420 nm. Wrinkled area percentage (WAP) or rutab spots area (RSA) were determined visually and expressed as the percentage of affected fruit.

The experiment was conducted based on a completely randomized design (CRD) with three replications. The data were analyzed with MSTAT-C (version 1.42) statistical package, and means compared by Duncan's Multiple Range Test (DMRT) at 0.05 probability levels.

RESULTS AND DISCUSSION

Khalal dates stored at 30°C had maximum weight loss (27%) and no major differences were seen between 5 and 15°C storage temperatures. However 1-MCP could reduce the fruit weight loss at 5 and 30°C but its effect was not significant (Fig. 1a). This observation is consistent with those previously reported for apricots (Fan et al., 2000) and ripe tomatoes (Will and Ku, 2002). Percentage weight loss of fruits increased with prolonged storage period and 1-MCP did not cause marked variations in weight loss relative to that of the control at different time intervals. The highest weight loss was obtained in control khalal dates, with values of 27.5% after 28 days of storage since it was 25.7% for fruits treated with 1-MCP (Fig. 1b). Weight loss is a physiological event caused by loss of water from the fruit surface to the surrounding atmosphere and loss of carbon on formation of CO_2 during respiration (Rizzo and Muratore, 2009). Loss of about 3.5, 6.1 and 20.9% of fruit weight during the eight days of storage at 5, 15 and 30°C respectively indicates the economic importance of storage temperature in trade of khalal dates.

The initial firmness measured at the start of experiment was 55.5 N. Application of 1-MCP was effective on delaying softening at all storage temperatures but its effect was higher and significant for fruits stored at 5 and 15°C (Fig. 2a). A typical fruit softening was observed in both control and 1-MCP treated fruits, but the reduction rate was higher for control fruits. The lowest and the highest firmness values at the end of 28 days storage were 7.7 and 10.2 N for control and 1-MCP treated fruits respectively (Fig. 2b). The effectiveness of 1-MCP in fruit firmness retention has been reported for many horticultural products such as guava (Bassetto et al., 2005), avocado (Feng et al., 2000), pear (Acuna et al., 2011) and plum (Valero et al., 2004). Khalal fruits that lost their hard and crisp texture have lower quality and price. Softening of fruit texture is related to activation of pectin decomposing enzymes. The delaying effect of 1-MCP on fruit softening probably resulted from the retardation of senescence processes due to inhibition of the ripening rate (Oz, 2011).

Browning is a limiting factor in the marketing of many horticultural products. It seems that browning at khalal date is a physiological process related to ripening, that starts in the fruit flesh, then affected surface color. Evaluation of fruit juice absorbance at 420 nm as browning index showed that this parameter was increased gradually in terms of storage duration, however the rate of browning was lower for fruits treated with 1-MCP but it was similar to control. Also the effect of 1-MCP in reduction of flesh browning was significant only at 5°C (Figs. 3a,b). Browning in many fruits is the results of chemical processes, involving polyphenol oxidase, catechol oxidase and other enzymes that create melanins and benzoquinone, resulting in a brown color (Yourk and Marshall, 2003).

Appearing rutab spots area (turning surface to brown color) is a common

physiological change during storage of date fruit at khalal stage. The result of this study showed that up to 4 days after storage, no significant differences were seen in relation to application of 1-MCP and storage temperature (Fig. 4a). With increasing storage time, and after 8 days of storage, RSA was increased rapidly for fruits stored at 30°C without differences between control and 1-MCP treated fruits. Fruits stored at 30°C completely turned to rutab, 16 days after storage when RSA were about 26 and 20% for fruits at 15 and 5°C respectively. The effect of 1-MCP in slowing appearing RSA was greater and significant at lower temperatures. In fruits stored at 5°C, after 20 days of storage, RSA were about 53 and 22% for control and 1-MCP treated fruits respectively. There is not any report on the physiology of khalal date fruit browning on the tree or after harvest but it seems that date fruit has a high potential of browning due to high level of phenolic compounds and their oxidative enzymes. The inhibitory effect of 1-MCP on appearing rutab spots can be related to slowing metabolism and corresponding biochemical reactions at ripening.

Wrinkled area percentage (WAP) is another important factor that affects quality of date fruit at khalal stage. As shown in Figure 4b, WAP was higher at 30°C storage temperature and it followed a similar pattern of fruit weight loss during storage. At different time intervals of analysis, 1-MCP did not have any significant effect on WAP; however, this parameter was slightly lower for 1-MCP treated fruits. Also up to 16 days of storage, WAP was between 6-13% for fruits stored at 5 and 15°C, since it was about 60% for fruits at 30°C. Storing at 5°C, could keep WAP to less than 18%, up to 24 days after storage and it can be understood from Figure 4b that no significant difference exists between 5 and 15°C storage temperatures at least up to 16 days after storage.

Total soluble solids (TSS), titratable acidity (TA) and their ratio are the main quality factors affecting taste and flavor. The TSS concentration significantly increased during the storage, from level at harvest of 43.00 to 60.36%, at the end of the experiment (Table 1). The increase in TSS was in correlation with RSA (r^2=0.86, data not shown). Khalal dates treated with 1-MCP did not exhibit any significant changes in TSS, but the effect of storage temperature on fruit TSS was significant and maximum value was recorded in fruits stored at 30°C (60.7-62.7% for 1-MCP treated and control fruits respectively). The effects of 1-MCP on soluble solids content have been studied by different authors in different horticultural crops. Moretti et al. (2002) reported that 1-MCP treated tomatoes had the same TSS as control. Similarly, Porat et al. (1999) and Fan and Mattheis (2000) observed that citrus fruits and lettuce treated with 1-MCP had no significant changes in soluble solids content. In contrast to rutab fruits produced from khalal dates (in this work), the TSS of rutab date fruits naturally ripened on the tree is more than 75% and, those have an acceptable quality. It is postulated that the immobile form of the ripening enzymes existing in fruits harvested at khalal stage, is in contrast with date fruits left on the palm till they turn naturally to rutab. Formation of some metabolites including soluble contents arises by releasing and activating the enzymes such as amylase, invertase, polygalacturonase and polyphenol oxidases (Saleem et al., 2005).

At harvest, the levels of titratable acidity (TA) calculated as malic acid was 0.13% and TA decreased gradually throughout the evaluation period. TA was slightly lower for fruits treated with 1-MCP and also a few differences were found between the storage temperatures in terms of titratable acidity (Table 1). Linear loss of acidity was consistent with Cai et al. (2006) reported for loquat fruit. Organic acids usually decline during ripening as they are respired or converted to sugars. Also decreasing the level of organic acids simultaneously with the development of rutab spots can be related to their reaction with reducing sugars to produce brown pigments (Lozano, 2006).

The effects of 1-MCP and three storage temperatures on pH changes of khalal date fruits are shown in Table 1. The pH values of treatments were increased from initial 6 to 7 during storage but differences between treatments were found to be insignificant and 1-MCP treatment was determined to be ineffective. These results are similar to those of Oz (2011) for persimmon fruit. Storage temperature affected fruit pH and maximum pH

was observed for fruits stored at 30°C.

Lightness which exhibits the black-white index of surface color affected significantly by 1-MCP treatment in fruits stored at 5°C and no changes were seen at 15 and 30°C storage temperatures (Table 2). This pattern was in accordance with flesh browning of khalal dates (Fig. 3a). Hershkovitz et al. (2005) reported the significant effects of 1-MCP on lightness of avocado fruit. Other surface color spaces including hue angle and chroma were not affected by 1-MCP. Increasing the storage temperature decreased the hue angle and minimum hue value recorded at 30°C. Chroma also reduced at higher storage temperatures and it reached to about 38 for fruits stored at 30°C (Table 2).

CONCLUSIONS

The date fruit of cultivar 'Barhee' has lost its astringency at khalal stage and is sold as a fresh fruit. This stage will last for a couple of weeks (Glasner et al., 2002). Fruit at khalal stage has limited shelf-life and generally is sold shortly after harvest and mostly in the local markets.

The general pattern of changes show that temperature is one of the most important factors affecting quality attributes of khalal dates and many studied quality parameters were affected by storage temperature. A high level of weight loss, flesh and surface browning and fruit softening at 30°C leads us to this issue that storing khalal dates at low temperatures is a critical point in its trade. Also comparison of two storage temperatures of 5 and 15°C showed no major differences in terms of effect on RSA, TSS, weight loss, lightness and WAP. This may be important to choose a lower storage temperature based on economic limitations. Treatment with 1-MCP was effective in slowing the firmness loss, weight loss and appearing RSA but its effect was evident mostly at 5°C. Evident correlations displayed between RSA and flesh softening, browning index, lightness and TSS postulated the biochemical changes occurring at ripening stage issued from texture degradation by means of activation of different enzymes. To understand better these biochemical changes, research dealing with and focused on the understanding of the processes involved might be proposed. Based on our results a conservative recommendation to minimize quality losses and more effectiveness of using 1-MCP would be to keep the khalal dates at lower temperatures during storage.

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Tables

Table 1. TSS, pH and titratable acidity in control and 1-MCP treated khalal dates at different storage temperatures (5, 15 and 30°C).

		Storage temperature (°C)		
		5°C	15°C	30°C
TSS (%)	Control	46.73 b†	48.58 b	62.71 a
	1-MCP	46.42 b	47.96 b	60.73 a
pH	Control	6.26 c	6.43 b	6.73 a
	1-MCP	6.46 b	6.50 b	6.66 a
Titratable acidity	Control	0.12 a	0.11 ab	0.10 b
	1-MCP	0.10 b	0.10 b	0.10 b

† Values with different letters are significantly different at P<0.05.

Table 2. Changes of lightness, hue angle and chroma in control and 1-MCP treated khalal dates at different storage temperatures (5, 15 and 30°C).

	5°C		15°C		30°C	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
Lightness	63.00b†	65.33a	62.64b	62.24b	55.69c	55.26c
Hue	81.37ab	81.74a	79.77ab	79.58b	72.83c	73.37c
Chroma	44.41b	44.38b	45.57ab	47.15a	38.51c	38.01c

†Values with different letters are significantly different at P<0.05.

Figures

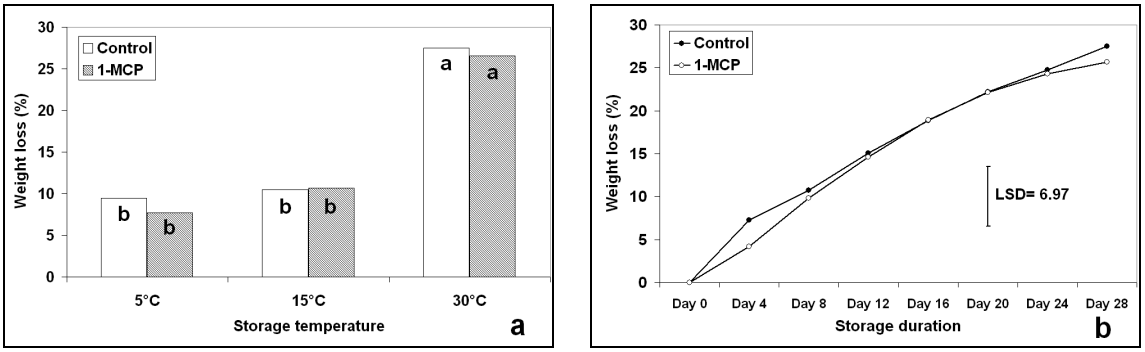

Fig. 1. The effect of 1-MCP in interaction with the storage temperature (a) and time of storage (b) on weight loss (%).

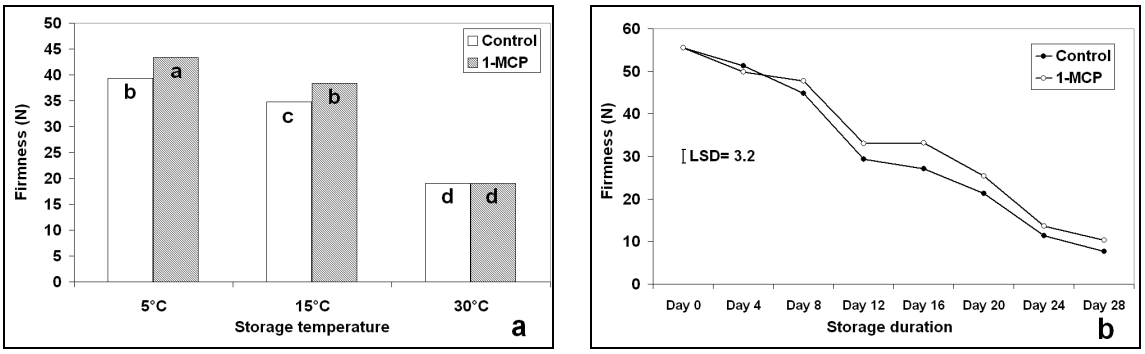

Fig. 2. The effect of 1-MCP in interaction with the storage temperature (a) and time of storage (b) on flesh firmness (N).

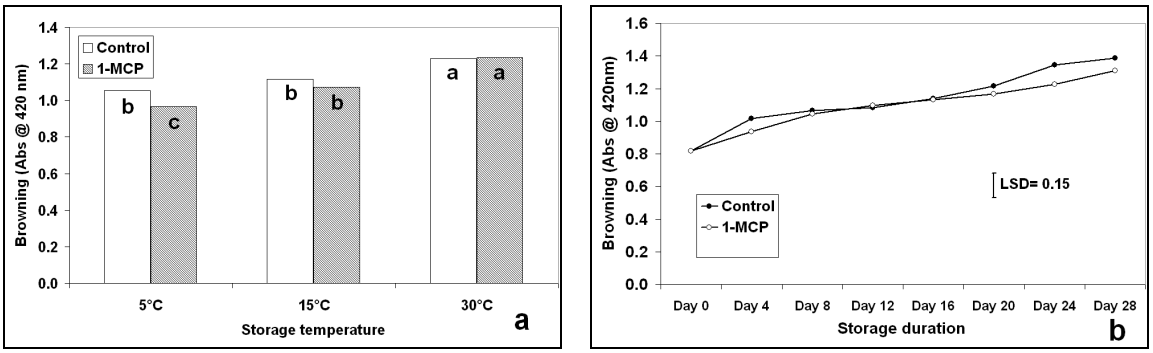

Fig. 3. The effect of 1-MCP in interaction with the storage temperature (a) and time of storage (b) on flesh darkening (absorbance at 420 nm).

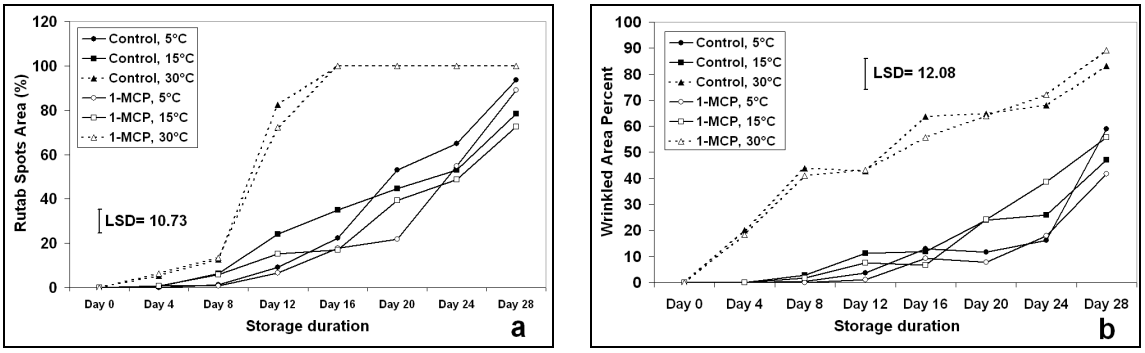

Fig. 4. The effect of 1-MCP in interaction with the storage temperature and time of storage on RSA (a) and WAP (b).

Dwarf Disorder in Tissue Cultured Date Palms - a Case Study

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Keywords: *Phoenix dactylifera*, micropropagation, *Arenipses sabella*, insecticide

Abstract

Tissue culture derived date palms (*Phoenix dactylifera* L.) are susceptible to many physiological disorders in the field. During the field evaluation of tissue culture derived date palms, the authors noticed sudden dwarfing symptoms in a few date palms belonging to the cultivars 'Succari', 'Sultana' and 'Anbara' at the flowering stage. The unnoticed affected palms produced little malformed fronds and face death at the end. In order to study the real cause for this dwarf disorder, a case study was carried out in our laboratory. One of the date palm cultivars, 'Succary', affected by this disorder was isolated and removed from the field for morphological and anatomical studies. While dissecting out the older leaves from the base towards the shoot tip many holes and furrows on the fronts were noticed. In few fronds the furrows deepen to the shoot meristematic region. Several insect larvae were also noticed in between the young fronds which were later identified as the larvae of the grater date moth *Arenipses sabella*. Some of the infected palms partially dissected out and treated with Benlate fungicide and Malathion insecticide in the field recovered from the disorder and produced normal fruits. Our study concluded that this dwarf disorder was caused by the greater date moth (*Arenipses sabella*) larvae followed by secondary fungal infection on the wound and this disorder occurs not only in tissue culture derived date palms, but also in seedling and offshoot derived date palms in Kuwait. This study also confirmed that the sudden dwarfing disorder occurred on the field grown tissue culture derived date palms are not due to genetic disorder but due to physiological reasons which could be controlled by treating the affected palm at the right time.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a tree of value and a major food crop of the Arabian Peninsula. The conventional propagation of the date palm is sexually through seeds and vegetatively through offshoots. Since the species is genetically heterogeneous, offshoot propagation was used for many generations to maintain the clonal genetic integrity of the selected cultivars. On the other hand, offshoot propagation is slow, expensive and prone to transmit insects and diseases. Tissue culture micropropagation technology was developed and successfully implemented to produce several thousands of young and uniform plants that were planted in orchards for production of dates (Sudhersan et al., 1993a,b; Sudhersan and Aboel-Nil, 2004).

Field observations indicated the true-to-type nature of the plants in terms of growth morphology and fruiting (Zaid and De wet, 2002; Sudhersan and Aboel-Nil, 2004). However, certain growth abnormalities or off-types were observed in some tissue cultured plants of few cultivars. The abnormalities found in micropropagated date palms are: changed morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, higher susceptibility to diseases, production of bastard offshoots (Zaid and Arias, 1999) or hapaxanthic axillary shoot formation (Sudhersan et al., 2001), hermaphroditism (Sudhersan and Aboel-Nil, 1999) delayed flowering time, pollination

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failure, abnormal fruiting, and seedless fruits. Generally these abnormal plants produced from tissue culture were known as off-type plants (Smith and Aynsley, 1995; Zaid and Al-Kaabi, 2003) or somaclonal variant (Larkin and Scowcroft, 1982). Among the off-types occurred in date palm, dwarfs are commonly noticed and reported. In addition to these date palm abnormalities, sudden dwarfing of date palms at the stage of flowering occurred in a few 3-5-year-old young tissue culture derived date palms.

The ability to propagate plants free from off-types through tissue culture is often dependent on the technique used for micro-propagation, nature of mother plant, type of growth regulators, type of explant, age of culture, medium composition, and culture incubation conditions. The off-types noticed at the culture level or greenhouse level can be easily eliminated before going to the customers. However, the sudden dwarfing of healthy tissue culture derived date palms in the field at the stage of flowering caused worries among the tissue culture researchers and farmers. Therefore, in order to find out the real cause for this sudden dwarfing syndrome, a case study has been carried out in the Biotechnology Department of Kuwait Institute for Scientific Research (KISR). The results of the study are reported herein.

MATERIALS AND METHODS

Plant Material

The tissue culture date palm orchards having 426 young palms of 29 different cultivars (24 female and 5 male) were used for the study. Palms affected by various physiological disorders were first identified in the date palm orchard. Few affected palms were dissected out using electric saw, hacksaw and sharp knives. Leaves were removed one by one from the base to the shoot tip. All the abnormalities observed on the fronds and stem were photographed and documented.

Insect Identification

The insect larvae observed from the affected palms while dissecting the shoots were collected in containers with ventilation for the experiments and insect identification. The insect larvae were fed by tender date palm fronds inside the laboratory and maintained at room temperature.

Experiment on Insect Larvae

An experiment was conducted using the larvae to correlate the symptoms observed on the affected palms and to confirm the causal agent for the disorders. For the experiment, 25 GA7 culture box with ventilation were used. In each box, a piece of 5 cm length young date palm frond and one insect larva were kept at closed conditions for 24 h. The changes on the fronds were observed and photographed. The insect larvae were fed by the young date palm fronds and maintained for the metamorphosis. At the pupa stage the larvae were maintained at 28±1°C until the insects came out from the pupa cover.

Treating the Dwarfs

The affected palms and the palms showing the symptoms of infestation were first treated with Malathion (2-5 ml/L water) followed by 1-2 % Benlate solution. Prior to the treatment with insecticide and fungicide, the infected fronds, basal leaf spines and older leaves were removed in order to make it easy to treat the palms. Treated palms were carefully maintained until the complete recovery from the disorder.

Field Study

A field study was carried out on the offshoot propagated palms and seed originated palms growing in farms and roadsides in order to confirm the sudden dwarfing syndrome occuring only in tissue culture derived date palms or both in tissue culture derived palms and seedling derived date palms. Several palms showing the similar disorders were identified and observed for several months. The morphological changes on

the affected palms were carefully observed and the details were documented. All the morphological changes and the symptoms observed from the affected palms were compared with the details collected from the tissue culture derived palms maintained at the KISR tissue culture orchard.

RESULTS

Among the 24 female and 5 male tissue culture derived date palms growing in the KISR date palm orchard, dwarfing disorder was noticed in 'Succari', 'Sultana' and 'Anbarah' (Table 1). The tissue culture derived date palms suddenly turned to dwarfs in the field showed many growth abnormalities (Figs. 1-6) on the vegetative and reproductive organs. The abnormalities noticed on the affected palms in the orchard were: 1. front malformation, 2. scratches and furrows on the leaf rachis, 3. absence of leaflets, 4. little leaves, 5. 'v' cut on leaf base, rachis, flower stalks and spathe, 6. leaf drying, 7. inflorescence without flowers, 8. inflorescence drying, 9. fruit bunch braking and 10. crown bending.

The dissected out infected palms showed furrows and holes on the young fronds, axillary shoots, inflorescences, fruit bunches and tender part of the stem that were filled with large quantities of "frass" (fibrous excrement) produced by small insect larvae. While dissecting the leaves of the affected palm, many insect larvae were noticed. The larvae were feeding on tissue between veins or ribs of the leaflets, rachis of the frond and leaf bases. Some of the furrows made by the larvae were tunneled from the leaf base into the tree tender part of the stem. The larvae enter the large terminal bud of the tree through the furrows which they made and fed on the tender leaf primordia causing malformations on the fronds and floral organs. In addition to insect damage, fungal infection was also noticed on the stem tender tissue (Figs. 7-9) which is secondary in origin.

Many small and grown up insect larvae about 4-5 cm in length were found feeding on the tender leaves and stem tissue while dissecting the palms that showed the dwarf symptoms. The larvae were collected and experimented with young date palm fronds showing similar symptoms such as: 1. scratches on the leaf rachis by eating the surface tissue, 2. silky web secretions with excretory materials and 3. holes and furrows on the frond that were noticed on the affected trees. By these observations the larvae collected from the abnormal date palm trees were identified as the causal agent for the sudden dwarfing disorder.

The larvae stayed active for more than a week (Figs. 10-11) time when the feed was available inside the ventilated container. Many small larvae were died when there is no feed while the larger ones made silky cover around by the secretions and turned into pupa. The pupa maintained at low temperatures stayed as it is for long time and the ones maintained at 28-30°C metamorphosed into adult insect (Fig. 12) after a week time. The adult insect came out through a small pore on one side of the pupa cover. The insect was identified as the date moth *Arenipses sabella*.

The affected palms treated with insecticide and fungicide solutions, and maintained in the field turned to produce normal fronds after 3 to 4 months time. The palms that reversed to normal condition produced normal fruits. The unnoticed palms produced malformed fronds and finally died. In some cases, the axillary shoots were activated during the death of the main shoot.

Some of the seed originated palms and offshoot derived palms growing for fruit production and landscape beautification also showed similar disorders that were observed in the tissue cultured date palms. Many such trees produced malformed fronds and showed crown bending. Palm trees showing stunted growth and bent crown with malformed fronds were commonly noticed along the road sides of Kuwait. Many such trees recovered automatically and other trees died after few months. In some cases when the apical meristem was damaged the axillary shoots were activated and branches were produced. During our field observation many palms once showed dwarf symptoms recovered to normal trees.

DISCUSSION

The tissue culture derived date palms which suddenly turned to dwarfs showed many abnormalities on the fronds and on the floral organs. All these abnormalities were created by the insect larvae. The leaves attacked by the larvae at the primordial stage showed malformed frond with irregular leaflets and scratches on the rachis and inflorescence axis. Later on secondary infection caused by the fungus through the wound affected the vascular tissue of the inner leaf primordia. Due to this secondary infection on the new leaf primordia, the elongation of the leaflets and the rachis was affected and finally the leaves became little leaves or died completely.

The 'v' shaped cuts and holes on the leaf base, rachis of the leaves, inflorescence stalks and spathes were also due to the larval attack on the leaf or inflorescence at the primordial stage. The small cut on margins by the larva at the primordial stage expanded when the leaf or inflorescence grow and appeared as 'v' shaped cut. Due to the cut at the inflorescence stack at the basal region some of the fruiting bunches were broken and dried. The sudden dwarfing of field grown palms made a severe loss to the farmers. Many researchers and farmers when they observed this disorder in tissue culture derived date palms thought that it is due to a genetic disorder.

Some of the previous reports on the abnormal tissue culture derived plants misled the farmers to think that the abnormality occurs only in tissue cultured derived trees. In micropropagated oil palm, three major abnormalities were observed and reported (Corley et al., 1986). All these abnormalities were commonly found in 2-3-year-old young palms and rarely in older palms (Williams and Thomas, 1970). The report on tissue cultured oil palm abnormalities created a loss of confidence in clonal oil palm. However, commercial scale clonal oil palm plantations produced by some laboratories proved that tissue cultured oil palms were normal, highly uniform and productive after 25 months of field transfer (Khaw and Ng, 1998) with the incidence of negligible abnormalities. The dwarf off-types occurring in tissue culture can be controlled by the culture techniques itself and even it can be roughed off at the early stages of development. Generally at the initial 1-3 years of vegetative growth of date palms, several reported abnormalities occurred in normal seedling palms (Leak, 1914), offshoot palms and tissue cultured palms (Sudhersan et al., 2001).

Many insects attack the floral and vegetative parts of the date palm trees (Stickney et al., 1950). At the initial stages of infestation by the larvae of greater date palm moth (*Arenipses sabella*) leaflets showed malformation on all or parts of the frond and stunting of the entire frond. The crown bending is due to the damage of the meristematic tissue and young fronts at one side of the growing point. The new leaf production and growth of the fronds on the other parts make the entire tree crown bend towards the point of insect or fungal attack. When the larvae attacked the basal region of single leaf primordia, that single frond showed malformation at maturity. Few months after insect infestation, fungal infection through wounds followed and extended into the terminal meristem. Axillary shoots that developed from buds at the axils of unaffected leaves develop into normal offshoots. On the other hand, if the insect infestation was not treated, offshoots may also get infested and show the symptom of frond malformation. Palms that show any sign of leaf malformation or death of a single leaf or presence of the frass of the insect on the leaves should be treated for insect infestation by spraying the entire tree with a systemic insecticide. Observations presented in this study cleared confusion over the cause of sudden dwarfing of some tissue cultured date palms in the field at the flowering stage.

Most of the reported abnormalities like leaf malformation, bastard offshoots (Zaid and Al-Kaabi, 2003), hapaxanthic axillary shoots (Sudhersan et al., 2001) and hermaphroditism (Sudhersan and AboEl-Nil, 1999) were not unusual and specific to palm derived from tissue culture, but common in young seedling palms, offshoot derived palms and tissue cultured palms. These abnormalities will disappear as they grow and develop. Tissue cultured palms developed through somatic embryogenesis with proper culture techniques and management were found to be true-to-type (Al-Shayji et al., 1994; Sudhersan and AboEl-Nil, 2004) and they are similar to the seedling palms in growth and

development. In our experience since 1989, plantlets produced from several date palm cultivars through somatic embryogenesis method and tasted the true-to-type fruits from them. Research documented that the tissue cultured palms are true-to-type of the mother palms and somaclonal variations can be induced or avoided through different culture techniques and management.

Most of these physiological disorders in date palms were reported (Djerby, 1983; Darley et al., 1960) as diseases of unknown origin in date palms. However, the present study confirmed the real cause for the date palm disorders such as frond malformation, 'v' cut, crown bending, terminal shoot bud drying and little leaf syndrome. The main cause for these disorders was the date moth *Arenipses sabella*, followed by the secondary infection of fungi through the wound caused by the larvae. In our study, mainly 'Succary' and 'Sultana' date palm cultivars were found to be more susceptible to *Arenipses sabella* larvae than other cultivars.

Date palms affected by the sudden dwarfing disorder could be changed to the normal condition through insect or pathogen control, transferring the affected palms to good soil and location, and cutting the roots of the fence trees growing around the affected palms. It is very important that tissue cultured palms need special care including regular irrigation, fertilization, weeding and good control over pathogens and insect pests as they are more susceptible at early stages of growth and establishment. As a control measure, chemical control is an inevitable solution until an alternative environmentally friendly method is found for the control of this date palm moth *Arenipses sabella.*

CONCLUSION

The present study confirmed that these abnormalities occur not only in the tissue culture derived palm but also in the seed and offshoot derived date palms, and cleared the confusion over the tissue culture method of date palm propagation. Many of the disorders of date palms such as: front malformation, 'v' cut, crown bending, stunted growth and terminal bud drying reported as diseases of unknown origin have been cleared by this study. Perhaps for the first time the causal agents for these disorders were identified and it was proven that these disorders of tissue culture derived date palms were not of genetic origin but physiological disorders caused by the larvae of *Arenipses sabella* followed by the secondary fungal infection. Date palms affected by dwarf syndrome could be changed to the normal condition through insect or pathogen control at the right time.

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Tables

Table 1. Date palm cultivars affected by sudden dwarfing in the field.

Date palm cultivar	No. of palms observed	No. of palms infected	% of dwarfing
Sultana	5	2	40
Succari	6	3	50
Anbarah	5	1	20

Figures

Fig. 1.Date palm showing dwarfing disorder.

Fig. 2. Frond malformations.

Fig. 3. Insect larval frass on the rachis.

Fig. 4. 'V' cut on inflorescence rachis.

Fig. 5. Axillary shoot showing dwarf disorder.

Fig. 6. Axillary shoots affected by the insect larvae.

Fig. 7. Insect larval attack on the shoot

Fig. 8. Dissected out shoot buds showing larval infection.

Fig. 9. Insect larvae collected from the infected palms.

Fig. 10. *Arenipses sabella*.

Somatic Embryogenesis Approach for Shoot Tips of Date Palm 'Zaghlool'

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Abstract

This study was conducted to develop a protocol for somatic embryogenesis in shoot-tip-explants of date palm 'Zaghlool'. Explants formed callus on MS media containing 50 and 100 mg/L 2,4-D or 50 mg/L picloram with 3 mg/L 2iP and 1.5 or 3 g/L activated charcoal. On MS medium with picloram, embryogenic callus was induced while on medium containing 2,4-D non-embryogenic callus derived. Formation of small yellow nodules was noted 8 months after disinfection on picloram enriched medium while white rigid callus was formed on MS with 2,4-D after 4-8 months. After nodule formation, calli were transferred on a MS medium with 10 mg/L 2,4-D, 3 mg/L 2iP and 1.5 g/L activated charcoal for 2-4 months for further development. After that, for somatic embryo development, calli were portioned in samples of 100 mg fresh matter and distributed on 12 regeneration media containing various concentrations of NAA, BAP and activated charcoal. In the first six weeks after transfer on these regeneration media callus mass increased and after six weeks until 18 weeks somatic embryos have been formed. Embryogenic callus mass and number of resultant embryos were significantly increased by NAA. The highest biomass was recorded on MS with 0.1 mg/L NAA with or without activated charcoal; moreover, the highest number of embryos was produced on this medium. Per 100 mg fresh matter of callus on this medium about 8 embryos derived while on hormone-free MS medium only 2 to 4 embryos have been formed. Biomass growth index was recorded in 6-week intervals. The growth index was the highest after transferring to regeneration medium with gradual reduction until 18 weeks. Therefore, after 18 weeks the embryos were transferred to the maturation medium. All somatic embryos matured and germinated on MS medium with 1 mg/L NAA, 1.5 g/L activated charcoal after 8-10 weeks. All shoots rooted on liquid MS with 1 mg/L NAA.

INTRODUCTION

Date palm (2n=36) 'Zaghlool' is an important soft fruity cultivar widely cultivated in Egypt, Jordan and recently also in Syria. The mean production of one tree is about 116 kg as reported by AOAD (Arab Organization of Agricultural Development). The annual date fruit production in Syria is about 4,000 tons (FAOSTAT, 2008) and falls short of the population demand estimated at about 200,000 tons. Nowadays, the government of the Syrian Arab Republic encourages culturing of date palm by increasing the number of nurseries especially in Palmyra and Deir Az-Zour. However, lacking tissue culture techniques in these nurseries and using only traditional methods (seeds and offshoots) for propagation is one of the essential obstacles. Propagation of date palm on a large scale can only be achieved by using tissue culture techniques. Date palm is propagated in vitro by two methods: the first method is somatic embryogenesis via embryogenic callus (Al-Khayri, 2005) developed from shoot tips, root tips, leaflets, or

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inflorescence. The second procedure is organogenesis which provides date palm buds that eventually give plantlets without passing through the callus stage (Al-Khateeb, 2006). Most research focused on date palm somatic embryogenesis reported use of 2,4-D in high concentration (100 mg/L) with 3 mg/L 2iP and 3 g/L activated charcoal (AC) to induce callus on shoot tips (Tisserat, 1979; Zaid and Tisserat, 1983; Al-Khayri, 2005). For callus initiation in 'Zaghlool', using 2.4-D at 10 mg/L (Bekheet and Saker, 1998; Bekheet et al., 2001; Abo-El-Soaud et al., 2002; Taha et al., 2003) or 100 mg/L 2.4-D (Mohamed et al., 2001; Abo-El-Soaud et al., 2002) with 3 mg/L 2iP is reported. Using picloram to induce callus was rather neglected in date palm tissue cultures. However, some researchers reported using picloram for callus induction in other *Arecacea* species such as *Phoenix canariensis* (Huong et al., 1999) or *Areca catechu* L. (Karun et al., 2004). Omar and Novak (1990) reported using picloram for growth of date palm callus cultures. In their research, the highest growth of callus was recorded by using 48.29 mg/L picloram in comparison with diacamba and 2,4-D. This callus was of high value after subculturing on hormone-free MS medium. Our preliminary experiments presented good results by using picloram in the course of date palm somatic embryogenesis protocols (Pinker et al., 2009; Ibraheem et al., 2009). Callus derived from zygotic embryos of 'Deglet Nour' by using 2,4-D produced the highest number of somatic embryos after subculturing on medium with 1 mg/L picloram (Pinker et al., 2009). Moreover, adding 30 mg/L picloram on in vitro leaflet segments from the Syrian cultivars 'Asabe Elarous', 'Barban' and 'Zahdi' was the optimal solution to obtain embryogenic callus (Ibraheem et al., 2009).

In date palm tissue culture there is only limited information aimed at determining the effects of auxins and cytokinins in interaction with other media components (Asemota et al., 2007).

The aim of our work is to develop an approach for somatic embryogenesis in date palm 'Zaghlool' by testing several auxins for callus formation and optimizing the regeneration of callus by investing different concentrations of NAA and BAP and the effect of activated charcoal.

MATERIALS AND METHODS

Plant Material and Preparation of Explants

Shoot tips of date palm 'Zaghlool' were separated from healthy offshoots (3-4 years old) of 5-7 kg in weight and about 50-70 cm in height grown in the Central Laboratory of Development of Date Palm Research at Giza, Egypt. Outer leaves were removed acropetally, exposing the hearts of the offshoots (15-20 cm length, 6-8 cm width) which were transported to Germany.

The outer leaves of the offshoot hearts were removed exposing the shoot tip region (3-4 cm length, 1-1.5 cm width) with 3-4 primary leaves which were placed in a chilled antioxidant solution consisting of 100 mg/L ascorbic acid and 150 mg/L citric acid, to prevent browning. The shoot tips were disinfected by immersing in 0.3% $HgCl_2$ with 3 drops of tween 20 for 5 min under agitation and then they were washed three times in sterile distilled water before dividing them to small squares (0.5-1 cm^2) which formed our initial explants.

Nutrient Media

The experiment was conducted with Murashige and Skoog (MS) mineral medium (1962). Other addenda to the MS medium were 2.5 mg/L thiamine, 0.2 mg/L biotin, 0.2 mg/L pyridoxine, 100 mg/L myo-inisitol and 100 mg/L glutamine. The pH value of the nutrient media was adjusted at 5.8. After pH adjustment, agar (Serva, Kobe I) was added to the nutrient medium at 7 g/L and sucrose at 30 g/L. The medium was distributed into glass tissue cultures vessels (Sigma-Aldrich© capacity: 100 ml) with 35 ml of the medium/vessel. The vessels were closed with Magenta® B-cap. (B8648) and autoclaved at 121°C for 15 min.

Callus Induction and Proliferation

For callus induction, shoot tip explants were exposed to MS media with 50 mg/L, 100 mg/L 2,4-D or 50 mg/L picloram and 3 mg/L 2iP. Activated charcoal was added to all media at 1.5 g/L and also at 3 g/L for MS medium with 100 mg/L 2,4-D. Shoot tips were subcultured every 4-6 weeks for 8 months, followed by transferring to MS medium with 10 mg/L 2,4-D, 3 mg/L 2iP, and 1.5 g/L AC for 12 weeks by subculturing every 4 weeks. Every treatment has 15 replicates and the experiment was repeated once.

Somatic Embryo Regeneration

For induction of somatic embryos, calli (100 mg pro vessel) were cultured on MS media with several concentrations of NAA (0, 0.1, 1 mg/L) or BAP (0, 1, 5 mg/L) alone or both growth regulators at 1 mg/L. Half of the media were supplemented with 1.5 g/L activated charcoal. Every treatment had 10 replicates and the experiment was repeated once.

Culture Conditions

Culture conditions depended on the stage of growth; in general, the explants were maintained in the growth room at 25±2°C and in darkness until occurrence of embryogenic calli. After that, the calli were exposed to light for 16 h daily at 35 µmol m^{-2} s^{-1} photosynthetic active radiation.

Data Collection and Statistical Analysis

Evaluation of the effect of auxin concentrations and type on shoot tip explants has been taken every 6 weeks by recording the swollen and callus forming explants.

To test the effect of NAA, BAP and AC concentrations on embryogenic callus growth, the fresh matter has been recorded in 6-week-intervals for all replicates. The growth index of biomass was evaluated as mentioned by Godoy-Hernández and Vázquez-Flota (2006) who reported that the accumulated biomass corresponds to the difference between the final and the initial masses. $GI=(W_F-W_0)/W_0$ where GI represents growth index, and W_F and W_0 represent the final and initial masses, respectively.

After embryo development occurred, the resultant embryos (longer than 5 mm) were counted per callus. The experiment was designed in a completely randomized design. The data were subjected to analysis of variance (ANOVA) and the means were separated by using Tukey test at 5% significance level.

RESULTS AND DISCUSSION

Callus Induction

In our experiments, the common medium for callus induction on date palm (100 mg/L 2,4-D, 3 mg/L 2iP, and 3 g/L activated charcoal) resulted in calli without ability to form somatic embryos (Table 1). Some calli induced on this medium developed only roots after transferring on the medium with 10 mg/L 2,4-D, 3 mg/L 2iP, and 1.5 g/L AC. This could be due to the long exposure period on the induction medium. The exposure period on 2,4-D medium was same as on picloram medium - 8 months, this period is similar to the period reported by (Bhaskaran and Smith, 1992; Vermendi and Navarro, 1996). However, regarding 'Zaghlool', it was much longer than the period reported by Bekheet and Saker (1998) and Taha et al. (2003) where the explants were exposed to 2,4-D medium for only 2 and 4 months respectively and the resulting calli were directly transferred to a regeneration medium. On the medium with 50 mg/L picloram, 3 mg/L 2iP and 1.5 g/L AC the explants were swollen after 6-10 weeks and yellowish aggregated callus tissue (Fig. 1A) appeared after 7-8 months on the upper surface of the explants. These aggregates developed 6-12 weeks later into white embryogenic nodular callus on MS medium with 10 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g/L AC. This type of callus originated from small white colonies on the surface of the medium. Abo-El-Soaud et al. (2002) observed that this callus type originated from single

meristematic cells in the callus tissue. A high amount of this white friable embryogenic callus appeared on MS medium with 10 mg/L 2.4-D, 3 mg/L 2iP and 1.5 g/L AC and some somatic embryos were induced as well on this medium (Fig. 1B). Our results agree with many reports focusing on 'Zaghlool' which reported using 10 mg/L 2,4-D to produce embryogenic callus.

Callus Proliferation and Somatic Embryo Regeneration

To evaluate the effect of NAA, BAP and AC on the further callus growth, 100 mg portions of embryogenenic callus were subcultured on various media (Fig. 2A). Callus continued to grow on all media compositions (Fig. 2A), even on hormone-free medium. The best treatment for callus development, however, was MS medium with 0.1 or 1 mg/L NAA only. After 18 weeks, from 100 mg callus, 5.5-6 g fresh matter involving callus and somatic embryos at all stages has been developed. Adding 1 mg/L BAP to the medium with 1 mg/L NAA did not further enhance callus growth. By adding BAP alone at 1 mg/L the final biomass was only about 4 g. Our results agree with those of Asemota et al. (2007). They referred that in date palm the addition of cytokinin to the culture medium containing near optimal levels of NAA did not further promote callus growth. Also Jones (1990) reported that oil palm cultures do not require exogenous cytokinins for growth. Adding activated charcoal did not affect the growth of callus (Fig. 2A). However, the texture of some calli and somatic embryos was superior on MS with activated charcoal than calli on media without activated charcoal (Fig. 1C,D).

The growth index (Fig. 3) was the highest in the first 6 weeks and the biomass of all calli increased 4-7 times. The highest biomass growth index was calculated on MS medium with 0.1 mg/L NAA and 1.5 g/L AC. In the second subculture, callus growth index decreased to 2-4.5 and it was only 0.5-1.5 at the third subculture. The correlation between somatic embryos number and weight of biomass was high and attained to R^2=0.795.

The number of somatic embryos did also increase by adding NAA to the medium. In the case of MS with 0.1 mg/L NAA, the mean of resulted embryos was 7.75 per initially 100 mg callus (Fig. 2B). Using medium free of plant growth regulators resulted in the lowest number of embryos (Fig. 2B). Although MS medium without plant growth regulators was reported for callus regeneration in several literatures (Tisserat, 1979; Mater, 1989), this medium did not enhance high somatic embryo regeneration in our experiments. Using NAA in small concentrations (0.1 mg/L) in callus regeneration medium was reported by Zaid and Tisserat (1983), Mater (1986) and Al-Maari (1995) inducing vigorous embryos from other date palm cultivars.

Our results on medium with 0.1 mg/L NAA were superior to those reported by Veramendi and Navarro (1996) who obtained 22 embryos larger than 5 mm per 0.5 g of callus of date palm in 4 months of culturing on hormone-free MS medium and to those of Bekheet et al. (2001) who reported inducing 15 embryos from 1 g callus on hormone-free MS medium.

Shoot Growth and Rooting

In our experiment, all embryos of 'Zaghlool' germinated by using MS with 1 mg/L NAA and 1.5 g/L activated charcoal (Fig. 1E) as already found in 'Deglet Nour' and 'Asabe El-Arous' (Pinker et al., 2009). On this medium, healthy shoots of 10-15 cm in length and with 3-5 leaves were observed after 2-3 months. These shoots could be rooted well after 6-8 weeks by using liquid MS medium with 1 mg/L NAA (Fig. 1F).

Bekheet et al. (2001) reported that only 60% of the embryos developed into complete plantlets on a hormone-free MS medium and Vermendi and Navarro (1996) reported even a germination rate of only 10%.

CONCLUSION

By using picloram in induction medium an effective system for developing embryogenic callus, high frequency of somatic embryo regeneration and its further

development could be established. The further improvement of this system by using liquid media is under study. Further physiological and histological studies on possible interaction of picloram and 2,4-D are recommended. Moreover, investigating the role of picloram in other date palm cultivars is desirable. For somatic embryos development and rooting, NAA plays an important role.

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Tables

Table 1. Responses of shoot tips on several plant growth regulator combinations after 11 months of initial culture, subculture every 4-6 weeks.

After 8 months on MS with	Followed by 3 months on MS with	Callus formation (%)	Somatic embryo formation
100 mg/L 2.4-d, 3 mg/L 2iP, 3 g AC	10 mg/l 2.4-D, 3 mg/L 2iP, 1.5 g/L AC	40	-
100 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g/L AC	10 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g/L AC	35	-
50 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g AC	10 mg/L 2.4-D, 3 mg/l 2iP, 1.5 g/L AC	30	-
50 mg/L Pic, 3 mg/L 2iP, 1.5 g/L AC	10 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g/L AC	40	+

Figures

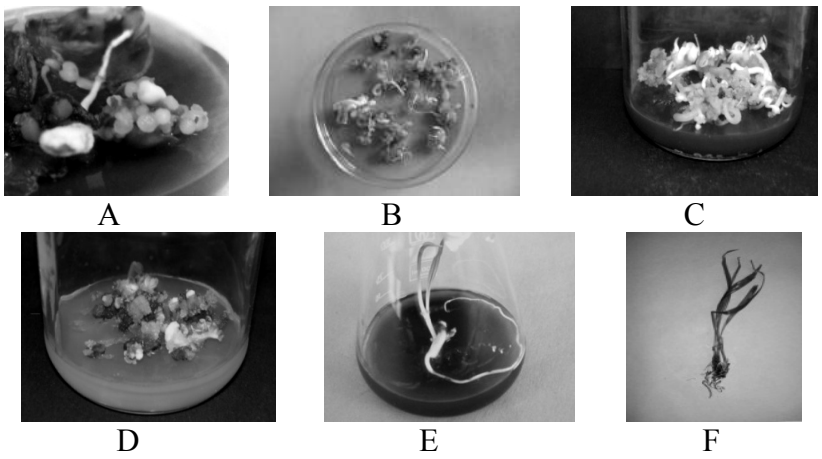
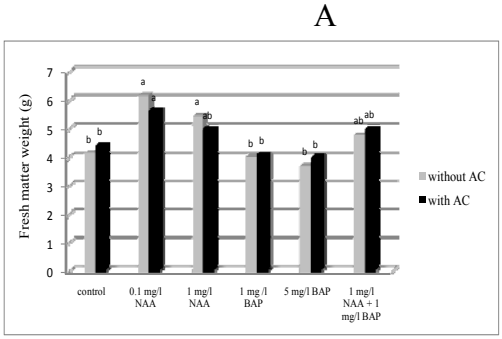


Fig. 1. A. Yellow nodules appeared on the explants surface after 7-8 months of initial culture on MS with 50 mg/L picloram, 3 mg/L 2iP and 1.5 g/L AC. B. Embryogenic callus 6-12 weeks later on MS with 10 mg/L 2.4-D, 3 mg/L 2iP, 1.5 g/L AC. C, D: The callus and somatic embryo texture on MS with AC (C) and without AC (D). E. Germination of somatic embryo on MS with 1 mg/L NAA with 1.5 g/L AC. F. Ready plant for acclimatization after rooting on liquid MS with 1 mg/L NAA.



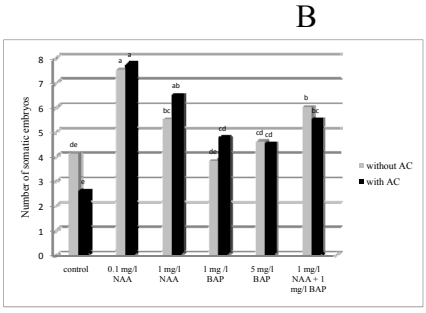


Fig. 2. Effect of NAA, BAP, and AC on biomass (A) and number of somatic embryos (B) after culturing embryogenic calli for 18 weeks (initial weight was 100 mg). Different letters indicate significant differences according to Tukey ($P<0.05$).

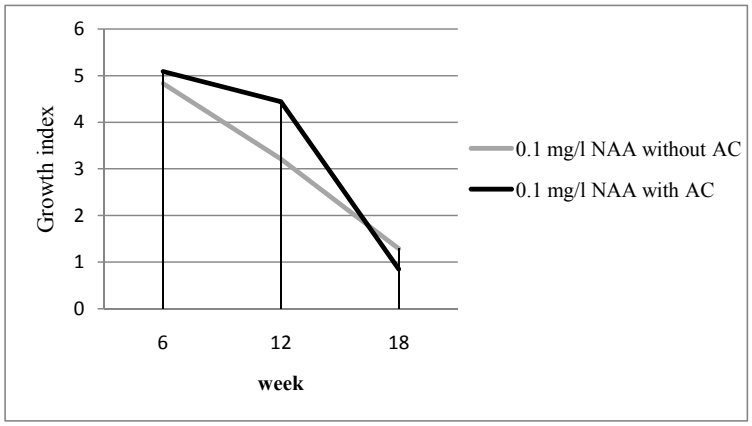


Fig. 3. The differences between biomass growth indexes depending on the periods of culture; for example, data were shown for MS with 0.1 NAA with and without activated charcoal.

Effect of Saprotrophic Fungi on Arbuscular Mycorrhizal Root Colonization and Seedlings Growth in Date Palm under Greenhouse Conditions

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Keywords: *Phoenix dactylifera*, mycorrhizal inoculation, biomass production, nitrate reductase activity, glutamine synthetase activity, saprobe fungi

Abstract

The interactions between the arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* (Gm) and *Glomus intraradices* (Gi) and the saprophytic fungi *Trametes versicolor* (Tv) and *Fusarium lateritium* (Fl) on arbuscular mycorrhizal root colonization and date palm growth were studied in greenhouse experiments. Overall, the results indicated that mycorrhizal inoculation improved date palm growth since biomass production was higher in mycorrhizal than in non-mycorrhizal seedlings. Nitrate reductase (NR) and glutamine synthetase (GS) activities were significantly higher in AMF plants compared to non-AMF plants. Date palm root colonization with Gm was significantly enhanced in the presence of the saprophytic fungus *Trametes versicolor*. The double inoculation with *Glomus mosseae* and *Trametes versicolor* showed higher biomass production and higher NR and GS activities. However, low NR and GS activities and biomass was produced by mycorrhizal plants when *Fusarium lateritium* was present. Thus, the effectiveness of the double inoculation in improving the growth of date palm varied among mycorrhizal and saprophytic fungi species, the interaction Gm/Tv being the most effective association to enhance date palm development.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are a principal component of the soil microbial biomass. When associated to plant roots these microorganisms improved plant growth, particularly through enhanced water and nutrient uptake (Smith and Read, 1997; Stutz et al., 2000). Several studies describing the positive effect of AMF on growth and development of pot-grown date palm seedlings were reported (Khaliel and Abou-Heilah, 1985; Oiahabi, 1991; Al-Whaibi and Khaliel, 1994; Meddich et al., 2004; Baslam et al., 2008, 2009a,b). In earlier studies we have shown that AM fungi allow for greater uptake of nutrients and play an important role in water relations and antioxidant metabolism thereby stimulating date palm growth under water deficiency (Baslam et al., 2008, 2009a,b). It is well known that soil microorganisms affect AM symbiosis function and development. Saprobe fungi are important and common components of rhizospheric soil where they obtain greater nutritional benefit from organic and inorganic compounds released from living roots together with sloughed cells (Alexander, 1977; Dix and Webster, 1995). Their metabolism may result in the production of substances that promote or inhibit the growth of other rhizospheric microorganisms (Fracchia et al., 1998; Garcìa-Romera et al., 1998). Several investigations indicating interactions between AM and saprotrophic fungi in the soil rhizosphere and in plant root colonization have been reported (Gryndler, 2000). Therefore, adverse, neutral and positive effects of saprotrophic fungi on AMF development and function have been observed (McAllister et al., 1997; Fracchia et al., 1998; Godeas et al., 1999). The aim of this work is to study the effect of the saprobe fungi *Fusarium lateranium* and *Trametes versicolor* on AM fungi root colonization and seedlings growth in date palm under greenhouse conditions.

MATERIALS AND METHODS

Seeds of *Phoenix dactylifera* L. collected from the date palm population of Marrakech were pre-germinated on wet sand. After three weeks, germinations were transplanted into plastic pots containing 1 kg of sterilized soil collected from the palm grove of Marrakech. Mycorrhizal inoculation was performed immediately (Inc1) or two months (Inc2) after germination. Two AMF strain *Glomus mosseae* (Gm) and *G. intraradices* (Gi) and two saprobe fungi species *Fusarium lateranium* (Fv) and *Trametes versicolor* (Tv) supplied by the Estación Experimental del Zaidín (Granada, Spain) were assessed. For each plant, 10 g of the corresponding inoculum (a mixture of rhizospheric soil from trap cultures containing spores, hyphae and mycorrhizal root fragments) was applied. The same amount of an autoclaved mixture of the inoculum was added to non-inoculated plants. For the inoculums preparation of saprobe fungi barleys seeds were inoculated with a thin slice of PDA (1×1 cm^2) with mycelia of a 14-days-old culture grown at 28°C. Each plant was inoculated with 6 barley seeds grown with the saprobe fungi at 28°C for 2 weeks. The experiment treatments were arranged in a complete randomized block design. Each treatment was replicated 20 times. After 120 days, root colonization (M) and mycorrhization frequency (F) were evaluated according to Trouvelot et al. (1986). Plant height and weight, and shoot and root weights were recorded. Foliar nitrate reductase activity (NR) was determined in vivo according to Heuer and Pault (1978). Leaf disc samples were infiltrated under vacuum in 10 ml of 50 mM phosphate buffer, pH 7.5, containing 0.1 M KNO_3 and 0.1% Triton X-100. After 5 min, the samples were transferred into an identical solution but without triton X-100 and incubated for 1 h at 28°C. For determination of the nitrite formed, 1 ml of the solution was supplemented with 0.25 ml of 1.5 M HCl, containing 1% sulfanilamide and 0.25 ml of 0.02% solution of N-(1-naphtylethylenediamine) dihydrochloride. The absorbance was measured at 540 nm and the NR activity was calculated from a standard curve established with $NaNO_2$ concentrations and expressed in produced µmol NO_2^- h^{-1} g^{-1} FW. For glutamine synthetase (GS) determination, 1 g fresh harvested samples were ground in liquid nitrogen with chilled mortars and pestles using 5 ml extraction medium containing: 50 mM Tris-HCl (pH 8.0), 1 µM Na_2MoO_4, 10 mM $MgSO_4$ $7H_2O$, 1 mM EDTA, 10 mM L-cysteine, 1% PVP-40. The extract was filtered through one layer of Miracloth, centrifuged at 10,000 g for 20 min (4°C), and the supernatant was used for the assay. GS activity was determined by a biosynthetic assay based on γ-glutamil hydroxamate synthesis (O'Neal and Joy, 1973). One unit of enzyme activity corresponded to 0.1 per min variation in absorbance at 540 nm. GS activity was expressed on a fresh weight basis (UE g^{-1}). Data were analyzed within the ANOVA modules of the Statistica software program (Statsoft, 1995). Analysis of variance and comparison of mean values by Newman-Keuls test were carried out for each sampling data.

RESULTS

Date palm seedlings roots of all AMF treatments (Gm, Gm/Fl, Gm/Tv, Gi, Gi/Fl and Gi/Tv) were colonized by arbuscular mycorrhizal fungi (AMF), ranging from 16 to 47% (Table 1). The mycorrhization frequency (F) and the level of root colonization (M) were greater in date palm seedlings inoculated immediately after germination (Inc1) than those inoculated two months later (Inc2). Root colonization and mycorrhization frequency were highest in seedlings inoculated in the presence of *Trametes versicolor*, while in the presence of *Fusarium lateranium* roots were less colonized with AMF than in the absence of saprobe fungi (Table 1). AMF improved plant growth since shoot height and plant weight (Table 1) and shoot weight (SW) and root weight (RW) were greatest in mycorrhizal plants (Fig. 1). Double inoculation with AMF and the saprophytic fungus *Trametes versicolor* significantly increased PW (Table 1) and SW and RW (Fig. 1) but had no significant effect on SH (Table 1) and SW/RW ration (Fig. 1). However, in the presence of *Fusarium lateranium*, obtained PW, SW and RW were lower than in the absence of saprobe fungi. Double inoculation Gm/Tv showed the highest PW (12.8 g), RW (4.2 g) and SW (8.6 g). Furthermore, higher nitrate reductase (0.31 µmol NO_2^- h^{-1} g^{-1})

and glutamine synthetase (211 UE g^{-1}) activities were observed in the Gm/Tv treatment (Fig. 2). Conversely, decreased nitrate reductase and glutamine synthetase activities and low biomass were produced by mycorrhizal plants when *Fusarium lateritium* was present (Fig. 2).

DISCUSSION

Our results showed that date palm roots were more colonized when AMF inoculation was performed immediately after germination. This result calls to reconsider the inoculating period of date palm seedlings. Indeed, in previous studies AMF inoculation was usually performed two months after germination corresponding to the appearance of fine roots (Oiahabi, 1991; Meddich et al., 2004). The effectiveness of double inoculation in improving roots colonization and thereby date palm growth varied among mycorrhizal and saprophytic fungi species. AMF fungi assessed (Gi and Gm) had positive effects when associated with the saprophytic fungus *Trametes versicolor*. The beneficial effect was more evident in seedlings double inoculated with *Glomus mosseae* and *Trametes versicolor.* Double inoculation with Gm and Tv showed higher root colonization and higher shoot and root fresh weight. Moreover, nitrate reductase (NR) and glutamine synthetase (GS) activities were significantly enhanced in the presence of Gm and Tv. However, decreased NR and GS activities and low biomass was produced by mycorrhizal plants when *Fusarium lateritium* was present (Fig. 2). Active contribution of mycorrhizae to NR and GS activities has been reported by several investigations (Smith et al., 1985; Azcon et al., 1992; Cliquet and Stewart, 1993; Tobar et al., 1994; Cuenca and Azcon, 1994; Ruiz-Lozano et al., 1996). This should result in higher biomass production by improving N uptake and assimilation by external hyphae of AM fungus. Higher GS activity is probably accompanied by increased amino acid synthesis (Smith et al., 1985; Cliquet and Stewart, 1993). Increased ammonia assimilation and Gln production and xylem nitrogen translocation has been reported in AMF inoculed maize plants (Cliquet and Stewart, 1993). The synergistic effect of saprotrophic and AMF fungi on AMF roots colonization and plant growth had been previously observed (Fracchia et al., 2000; Arriagada et al., 2004). The action of the saprobe fungi on mycorrhizal plants was very variable depending on AMF and saprobe fungi species (Arriagada et al., 2004; Vogel-Mikus et al., 2005). Furthermore, results from pots experiments indicated that saprotrophic fungi can affect AMF development and function when AMF are inside the root (McAllister et al., 1997). García-Romera et al. (1998) reported that AM colonization and plant growth was substantially increased in the presence of the saprophytic fungi *Fusarium oxysporum*-126. In conclusion, this study highlighted a positive response of date palm seedlings to the double inoculation with Gm and Tv. This positive effect is greater when inoculation occurs immediately after germination.

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Tables

Table 1. Effect of double inoculation with AM fungi, *Glomus mosseae* (Gm) and *G. intraradices* (Gi), and saprotrophic fungi *Fusarium lateranium* (Fl) and *Trametes versicolor* (Tv) immediately after germination (Inc1) or two months later (Inc2) on root colonization (M) and mycorrhization frequency (F) and shoot length (SL) and plant weight (PW) in date palm seedling under greenhouse conditions.

Inoculation treatment	Inoculation period	Shoot height	Plant weight	F	M
T		32.3±2.10	3.20±0.43	0	0
Gm	Inc1	34.5±1.10	5.60±0.26	21.4±1.76	2.20±0.08
	Inc2	29.3±0.50	8.10±0.51	26.1±1.14	13.60±1.61
Gi	Inc1	35.0±1.10	4.33±0.21	16.6±1.02	1.70±0.03
	Inc2	29.1±1.30	7.00±0.52	21.8±2.01	19.00±0.20
Gm/Tv	Inc1	34.5±1.05	7.67±0.35	40.0±3.33	6.70±0.88
	Inc2	33.9±1.41	12.8±1.70	47.1±2.88	27.70±1.77
Gm/Fv	Inc1	27.3±2.43	3.17±0.30	26.7±1.18	5.20±1.91
	Inc2	29.2±2.54	3.50±0.80	27.3±1.30	11.40±0.86
Gi/Tv	Inc1	34.5±1.91	5.28±0.28	36.7±2.40	8.00±1.18
	Inc2	33.8±1.42	10.53±0.77	45.8±1.35	21.20±0.10
Gi/Fv	Inc1	28.2±1.53	4.52±0.26	34.4±1.70	1.60±0.59
	Inc2	32.1±1.12	4.51±0.38	16.2±1.79	1.20±0.03

Figures

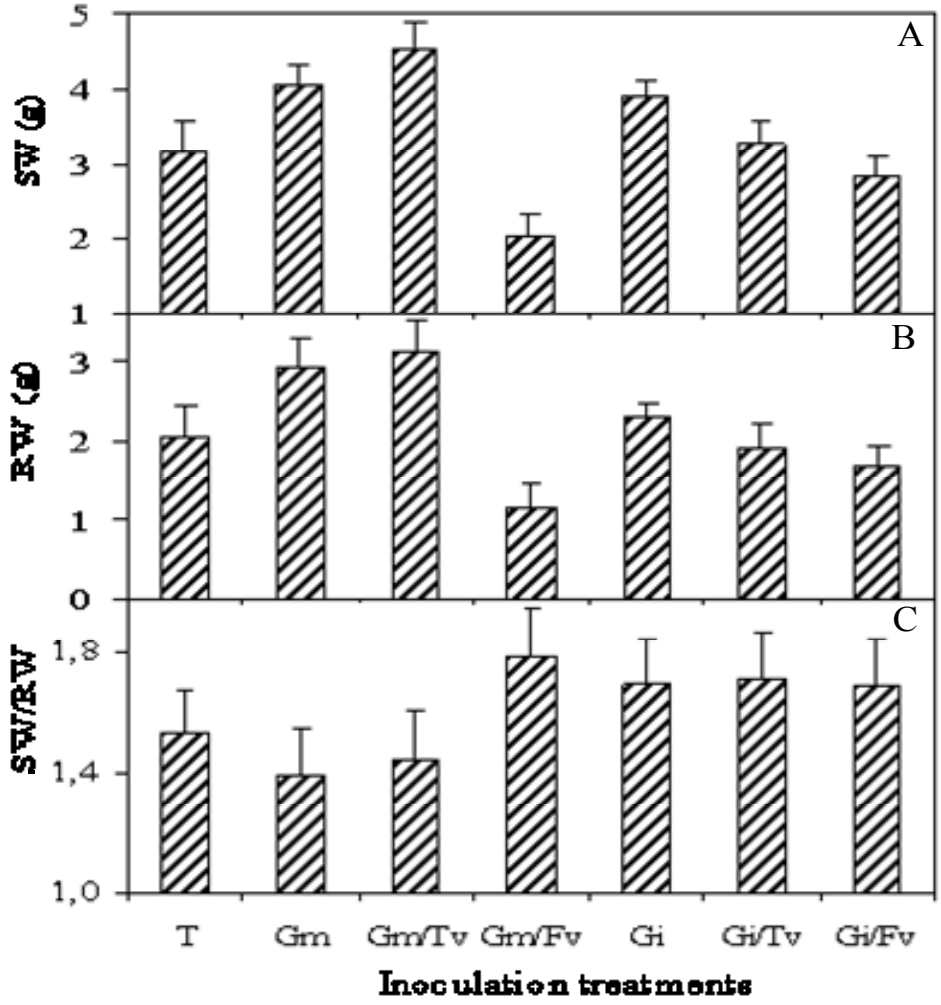

Fig. 1. Effect of double inoculation with AM fungi, *Glomus mosseae* (Gm) and *G. intraradices* (Gi), and saprotrophic fungi *Fusarium lateranium* (Fl) and *Trametes versicolor* (Tv) on shoot weight (A) and root weight (B) and shoot weight on root weight ratio (C) in date palm seedling under greenhouse conditions.

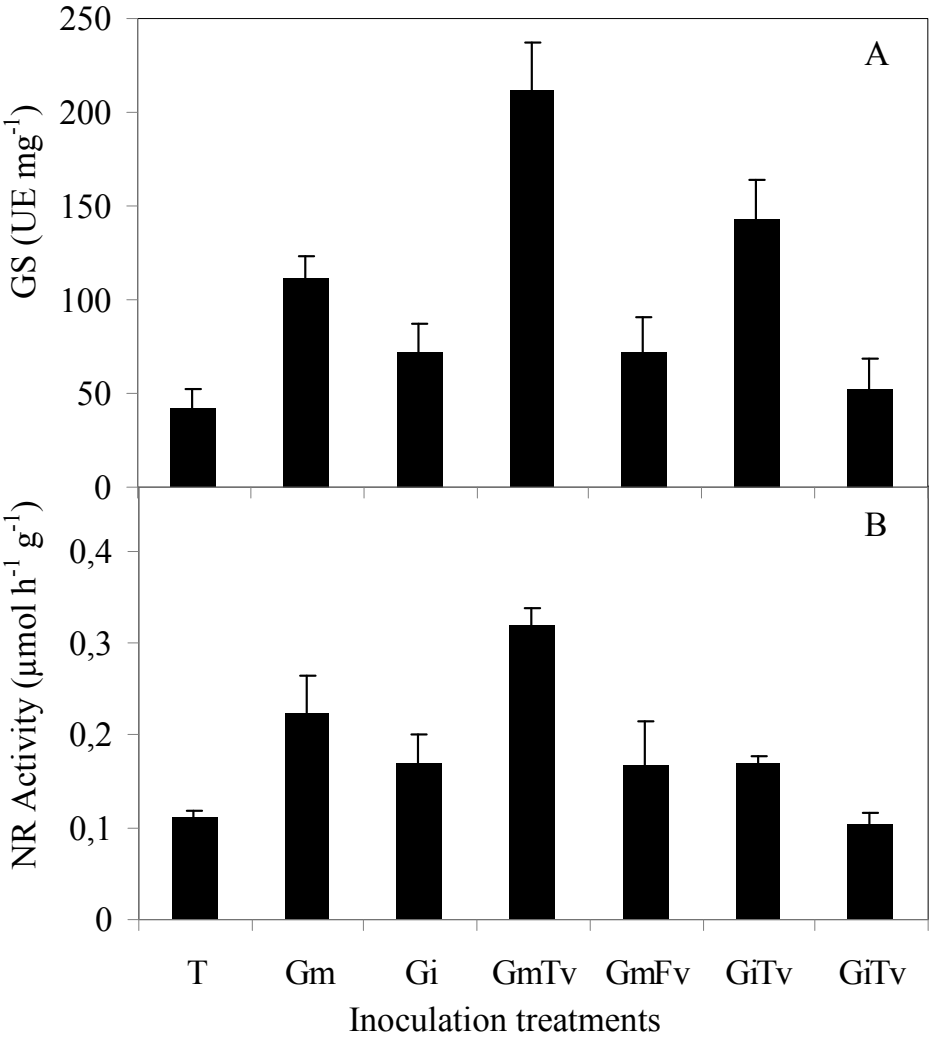

Fig. 2. Effect of double inoculation with AM fungi, *Glomus mosseae* (Gm) and *G. intraradices* (Gi), and saprophytic fungi *Fusarium lateranium* (Fl) and *Trametes versicolor* (Tv) on glutamine synthetase (A) and nitrate reductase (B) activities in date palm seedling under greenhouse conditions.

Effects of Thinning Methods on Date Bunch Fading Disorder at Pollination and Kimri Stages

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Keywords: date fading disorder, bunch thinning, 'Kabkab', pollination and kimri stages

Abstract

Date bunch fading disorder is one of the most important problems for date growers in southern Iran. Therefore, the effects of time and methods of bunch thinning were studied with a split plot experiment in Randomized Complete Block Design with 4 replications during 2003-2005. The treatments were comprised of thinning time at two levels (pollination and kimri stages) as the main plots and thinning methods at five levels (control, cutting back 10 and 30% of bunch tip, cutting back 10 and 30% of central strands) as subplots. The experiment was carried out on the 'Kabkab' cultivar in Bushehr province. The results showed that thinning treatments caused significant decrease (α=0.05) in fading disorder in experimental cultivars. The minimum percent of fading disorder in 'Kabkab' was observed when cutting back 30 and 10% of bunch tips and 30% of central strands, respectively. Time of thinning showed no significant effect on any of the characteristics. The interaction of time and methods of thinning treatments on fading disorder were significant at 5% level. Also, increasing of weight, length and fruit diameter were seen after bunch thinning. Thinning 30% of bunch tip in 'Kabkab' caused significant increase in pulp to seed ratio in comparison with control and other treatments. TSS was increased after bunch thinning significantly. Time and thinning methods had no significant effect on pH, ripening time and humidity percent.

INTRODUCTION

Date palms are dioecious, monocotyledon and permanent plant from the *Palmacea* family. Date is the most important income resource, food security, improvement sustainability and nutrient supply in date proceeding regions (Pezhman et al., 2005). Fading disorder is the most important factor that has decreased date's production in Iran in recent years. It was first reported from Kerman province and gradually distributed to the other main date growing areas. Pezhman et al. (2003) reported that the disorder occurs very suddenly and rapidly from khallal to rotab stage conversion, causing fade and eventually drought of date fruits as shown in Figure 1.

Many factors have been assumed to have effects on fading disorder such as fungi spores, bacteria, overloaded fruit, no tinning and climatical changes (Karampour and Pezhman, 2004; Mirzaee, 2001) but none of these factors was proven to be the main one (Pezhman et al., 2005). Some field reports showed that weather fluctuations, especially high temperature and blowing hot winds, would have influence on development of this disorder (Alavi, 2000). It was reported that thinning and covering of date bunches reduced fading disorder in 'Kabkab', 'Mozafatee' and 'Khassi' cultivars (Pezhman, 2003).

MATERIALS AND METHODS

A study was done to determine the effects of time and methods of bunch thinning on fading disorder in the Iranian Date Palm and Tropical Fruits Research Institute. The study was carried out with split plot experiment in Randomized Complete Block Design with 4 replications during 2003-2005. The treatments comprised of thinning time at two levels (pollination and kimri stages) as the main plots and thinning methods at five levels

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(control, cutting back 10 and 30% of bunch tip, cutting back 10 and 30% of central strands) as subplots. The experiment was carried out on the 'Kabkab' cultivar in Bushehr province. Each tree was taken as an experimental unit. Finally, 40 trees with the same age and size were selected in each region. The nominated gardens had shown fading disorder in previous years. After the occurrence of disorder in each region, repetitive observations were carried out to determine the damage percent in each tree. The percent of affected fruits was calculated by picking up 50 date fruits randomly from each palm and then multiplying the number of faded fruits by two. Any changes in the damage percent were notified within a specified bunch and its fruits in a tree (Fig. 2). Furthermore, some qualitative and quantitative characteristics of fruits such as pH, TSS, pulp to seed ratio and fruit weight, length and diameter were measured, too. The data were analyzed with MSTATC and the means were compared with Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

The results showed that thinning caused significant decreases (α=0.05) in fading disorder in experimental cultivars. The minimum percent of fading disorder in 'Kabkab' was observed when cutting back 30 and 10% of bunch tips and 30% of central strands, respectively (Table 1).

Time of thinning showed no significant effect on any of the characteristics. The interaction of time and methods of thinning treatments on fading disorder were significant at 5% level. Also, increasing of weight, length and fruit diameter were seen after bunch thinning. Thinning 30% of bunch tip in 'Kabkab' caused significant increase in pulp to seed ratio in comparison with control and other treatments. TSS was increased after bunch thinning significantly. Time and thinning methods had no significant effect on pH, ripening time and humidity percent.

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Tables

Table 1. The effect of time and method of bunch thinning on some date characteristics.

Thinning time	10% bunch tip	30% bunch tip	10% central strands	30% central stands	Blank	Mean
			Fading disorder percent			
Pollination	10.9ab*	9.1a	15.6d	14.1cd	20.6e	14.1A
Kimri stage	13.0bcd	8.8a	10.1ab	11.0abc	20.6e	12.7A
Mean	12.0B	9.0A	12.9B	12.6B	20.6C	
			Fruit weight			
Pollination	11.1bc	13.0a	10.8bc	12.3a	10.4c	11.5A
Kimri stage	11.1bc	12.3a	11.4b	12.6a	10.4c	11.6A
Mean	11.1B	12.7A	11.1B	12.4A	10.4C	
			Fruit length			
Pollination	37.2bc	39.6a	36.4c	38.8ab	36.0c	37.6A
Kimri stage	37.5bc	39.4a	37.3bc	39.2a	36.0c	37.9A
Mean	37.3C	39.5A	36.9BC	39.0A	36.0C	
			Fruit diameter			
Pollination	23.8bcd	24.9a	23.5cd	24.4abc	22.1e	23.8A
Kimri stage	23.4d	24.7ab	23.6cd	24.7ab	22.1e	23.7A
Mean	23.6B	24.8A	23.6B	24.5A	22.1C	
			Pulp to seed ratio			
Pollination	14.3b	16.3a	14.9ab	14.3b	14.3b	14.8A
Kimri stage	13.4b	15.0ab	14.1b	14.8ab	14.3b	14.3A
Mean	13.9B	15.7A	14.5B	14.6B	14.3B	
			Total soluble solid (TSS)			
Pollination	80.0abc	80.5abc	79.0c	82.4ab	78.3c	80.0A
Kimri stage	79.5bc	82.5ab	81.1abc	82.9a	78.3c	80.8A
Mean	79.8BC	81.5AB	80.0BC	82.6A	78.3C	
			Yield			
Pollination	30.1ab	31.0ab	34.2a	32.6ab	34.8a	32.5A
Kimri stage	26.9b	31.1ab	30.6ab	31.1ab	34.8a	30.9A
Mean	28.5B	31.3AB	32.4AB	31.4AB	34.8A	

* Means in each column or row with the same capital or small letter are not significantly different at 5% level.

Figures

Fig. 1. Showing the damage of a faded bunch and its fruit.

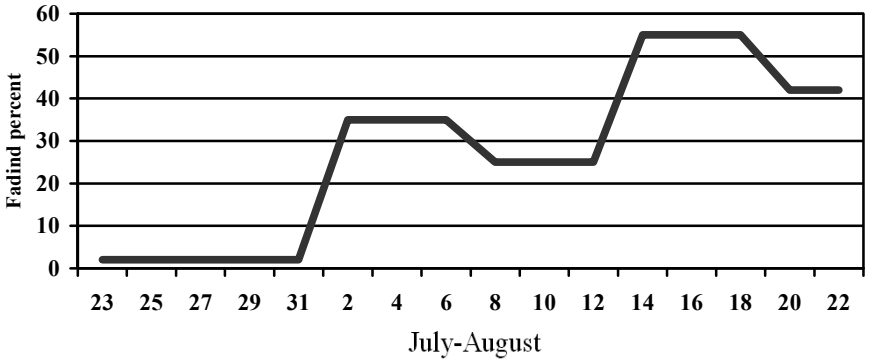

Fig. 2. Fading disorder damage development over time (Shirazi, 2008).

GC/MS Screening of Volatile Compounds Profiles from Healthy and Brittle Leaf Disease (BDL) Affected Date Palm (*Phoenix dactylifera* L.) - a Comparative Study

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Keywords: Algeria, *Phoenix dactylifera* L., brittle leaf disease, volatile compounds, fatty acids, GC/MS, screening

Abstract

The "brittle leaf disease" or "BLD", that appeared some years ago in north Africa, is one of the most destructive diseases of date palm (*Phoenix dactylifera* L.). In the present work we screened, for the first time, by gas chromatography-mass spectrometry (GC/MS), for changes in the volatile compounds profiles from healthy and BLD-affected date palm leaves. Chromatographic analysis showed that lipids and hydrocarbons composition of leaves change significantly. Indeed, a total of 43 volatile compounds including: fatty acids, hydrocarbons (aliphatic and aromatic), phenols and ketones, were identified in BLD-affected leaves, versus only 26 in healthy ones. Our results also revealed that n-Hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrien-1-ol, and octadecanoic acid, increase significantly in BLD-affected leaves; however, we registered a decrease in 1-hexadecene, 9-octadecyne and tocopherol contents. These results suggest implication of lipids (fatty acids in particular) in the plant defence reaction. So, further investigations must be carried out in the aim to check this assumption and to acquire better understanding concerning the date palm response to BLD.

INTRODUCTION

Since antiquity, the date palm (*Phoenix dactylifera* L.) represents an important crop in the desert regions of the Arabian Gulf peninsula and north African countries. This species, which is a long lived dioecious monocotyledon, belongs to the *Arecaceae* (*Palmae*) and constitutes the most important basic dietary component and income for people in the arid regions. It also plays an essential ecological role in adaptation to desert climate. Unfortunately, the date palm can be affected by many diseases some of which constitute a true threat for palm groves. One of the most destructive diseases of date palm (*Phoenix dactylifera* L.) is undeniably the wilt caused by the "brittle leaf disease" (BLD). The impact of this new pathology is very serious in north Africa, especially in Algeria and Tunisia where losses are increasing and may be a threat for palm-groves around the world. For that reason, the European and Mediterranean Plant Protection Organization (EPPO) Secretariat decided, in 2003, to add this disease in to the EPPO Alert List. Currently, more than 40,000 date palm trees have been destroyed in Tunisia (Nemsi et al., 2007) and many others have already died and have been removed in Algeria. Symptoms of the BLD first begin on the newly emerging palms. The leaves become chlorotic (yellow), small, and weak looking. As the disease progresses, fronds will emerge withered in appearance, dwarfed, distorted and leaflets become brittle, twisted, frizzled and shrivelled; that gives the name "brittle leaf disease" (Triki et al., 2003) to this affliction. According to Latreche (2008), serious changes affect the cell wall chemical

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component balance (decrease of cellulose and increase in lignins) which leads to foliage soft with the touch and breaking. Other observations and measurements reveal many modifications induced by the BLD on the date palm leaflets. The results indicate changes at morphological and histo-anatomical levels. These symptoms consist in serious cellular alterations that lead to necrosis. However, mineral element quantification at plant organ levels reveals high K^{+} accumulation and Mn^{+2}, Fe^{+2}, Zn^{+2} and Na^{+} decrease. In order to understand better the phenomena governing the interaction between the date palm and BLD, of which up to date the causal agent is still unknown, more precise studies are needed, especially with regard to lipids' and volatile compounds. Within this context, the aim of the current study was, thus, to screen for volatile compounds composition in the BLD-affected leaves.

EXPERIMENT

Plant Material

Diseased samples and healthy controls ones were collected from Tolga, Lichana and Bouchagroune palm groves (Biskra) located at 450 km in the south-east of Algeria (34°40'-35°00'N and 5°30'-5°55'E). The mature and fully expanded leaves were sampled. From each leaf, three leaflets were taken from the central part and were combined for one sample. Samples were then labeled and stored immediately in liquid nitrogen until use. All assays were performed with leaflet samples of the cultivar 'Deglet Nour'.

Extraction Procedure

The vegetal samples were washed in tap water to remove all dust. Samples were pulverized and pestled in a mortar and finally homogenized in an analytical mill. 40 g of leaves were extracted twice with 100 ml of acetone (GC grade) successively by shaking for 4 h on a mechanical horizontal shaker, followed by sonication at 50 KHz for 40 min at room temperature. The obtained crude acetonic extracts (CAE) were combined and filtered with suction using a Büchner funnel N°5 in order to eliminate the suspended particles. The CAEs were then concentrated with a rotary evaporator at 56°C to 1 ml approximately and subjected to second filtration through 0.45 µm Sep-Pak filter before the GC/MS analysis.

Gas Chromatography/Mass Spectrometry Analysis

Analysis of leaves' volatile compounds were performed by GC/MS. Samples were analyzed by a Hewlett Packard 5973 mass selective detector interfaced with a Hewlett Packard 6890 gas chromatograph, equipped with a computer workstation (Vectra 487/33VI Heweltt-Packard). The injector port and GC/MS interface were kept at 250 and 280°C, respectively. Separations were carried out on a fused silica capillary column (30 m×0.25 mm i.d.) coated with cross-linked 5% phenylmethylsiloxane (film thickness 0.33 µm). Helium was the carrier gas with a flow rate of 1 ml/min (average velocity= 59 cm/s). Samples (5 µl) were injected into the GC injection port using a splitless mode. Column temperature was increased from 60 to 250°C at 6°C/min after 2 min at 60°C, then held at 250°C for 20 min. Selected-ion monitoring was performed using the electron-ionization mode at 70 eV with the ion source maintained at 250°C. Identification of volatile compounds was achieved using their mass spectrum (MS) and retention times (TR).

RESULTS AND DISCUSSION

Profiles of leaves' volatile components from healthy and BLD-affected date palm are shown in Figure 1. The GC/MS analysis of the crude acetonic extracts (CAEs) revealed the identity of 51 molecules (Table 1). In healthy leaves, a total of 26 volatile compounds including mainly: lipids (55.41±4.72%), hydrocarbons (23.92±3.23%), phenols (7.81±0.53%) and ketones (1.80±0.33%) were identified. However, the profile of

BLD-affected leaves was richer and revealed others volatile compounds. Indeed, 43 molecules containing: lipids (81.73±2.87%), hydrocarbons (8.91±1.43%) and phenols (0.88±0.08%) were identified. Minor amounts of alcohol and ketones were also present, but these amounted to less than 1% of the total ion chromatogram.

On the other hand, BLD-affected leaves had a higher percentage of total saturated and unsaturated fatty acids (60.75±1.86%) than healthy ones (20.74±1.47%) and also contained C16:1ω-5 and C22:0 which were not detected in healthy leaves. Our results reveal also that amounts of n-hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrien-1-ol, and octadecanoic acid, increase significantly in BLD-affected leaves (31.10, 20.62, 19.90 and 7.96% respectively) when compared with those of healthy ones (12.49, 1.48, 16.75 and 3.71%, respectively).

These results, show clearly a serious disruption in fatty acids composition and volatile components of *Phoenix dactylifera* L. affected by the brittle leaf disease. The reasons for these changes are not yet clear, but they may be related with the plant defense reactions in which lipid metabolic pathways, those of fatty acids in particular, play an important role. Indeed, several discoveries have demonstrated direct roles for fatty acids and their breakdown products in inducing various modes of plant defenses (Huang et al., 2003; Yaeno et al., 2004; Tumlinson and Engelberth, 2008; Küpper et al., 2009). Both 16- and 18-carbon fatty acids participate in defense to modulate basal, effector-triggered, and systemic immunity in plants (Kachroo and Kachroo, 2009).

Studies of fatty acid metabolic mutants also revealed an active signaling role for the cuticle in plant defense. Indeed, to penetrate into the underlying epidermis, which is the outer protective layer composed of polyesters of C_{16} and C_{18} hydroxy fatty acids, fungus used enzymes hydrolyzing plant cuticles. It has been reported that degradation products from the cuticle could activate defense reaction in the host plants. In the present experiment, glycerol (0.66±0.10%) and 9-octadecenamide (0.39±0.04%) were only registered in the affected plant. According to Kim et al. (2006) exogenously treated PepEST protein on the unripe pepper fruits decomposed the cuticles of the fruits and released glycerol and 9-octadecenamide. These results suggest thus, a possible involvement of lipids, fatty acids in particular, in the induction of the date palm defense reactions against BLD.

CONCLUSION

The aim of this study was to know more about the physiological responses of the date palm (*Phoenix dactylifera* L.) when affected by brittle leaf disease (BLD). The causal agent of this pathology which assumes alarming proportions especially in Algeria and Tunisia is until now unknown. The screening carried out by gas chromatography-mass spectrometry (GC/MS) showed, for the first time, changes in the volatile compounds profiles from healthy and BLD-affected date palm leaves. The obtained results revealed that lipids' amount, saturated and unsaturated fatty acids in particular, increase significantly. These results suggest, thus, implication of lipids in the plant defence reaction. So, further investigations must be carried out in the aim to check this assumption and to acquire better understanding concerning date palm response to BLD.

Further investigations concerning the physiological response of the BLD-affected date palm, particularly the phenolic compounds, cellular membrane permeability and enzymatic activity, are in progress.

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Tables

Table 1. Volatile compounds identified in healthy and BLD-affected date palm leaves.

N°	Compound	Area (%)	
		Affected leaves	Healthy leaves
1	p-Xylene	0.22±0.02	-
2	Glycerol	0.66±0.10	-
3	Cyclopropane, 1-ethyl-2-heptyl-	0.08±0.01	-
4	Benzene, 1,3,5-trimethyl-	0.35±0.06	0.33±0.06
5	Benzene, 1-methyl-3-propyl-	0.17±0.08	-
6	Benzene, 1-methyl-2-propyl-	0.15±0.05	-
7	Benzene, 2-ethyl-1,4-dimethyl-	0.10±0.03	-
8	Benzene, 1-ethyl-2,4-dimethyl-	0.48±0.09	0.40±0.06
9	Benzene, 1,2,3,5-tetramethyl-	0.19±0.06	-
10	Benzene, 1,2,4,5-tetramethyl-	0.26±0.08	0.50±0.09
11	1H-Indene, 2,3-dihydro-5-methyl-	0.12±0.02	-
12	Benzene, 4-ethyl-1,2-dimethyl-	0.16±0.06	-
13	Naphthalene	0.78±0.21	1.13±0.17
14	1-Dodecene	0.39±0.11	0.56±0.12
15	3-Dodecene, (E)-	0.11±0.01	-
16	2-Dodecene, (Z)-	0.12±0.03	-
17	1-Tetradecene	0.73±0.18	1.01±0.19
18	3-Tetradecene, (Z)-	0.23±0.02	-
19	1,6-Anhydro-.beta.-D-glucopyranose	-	2.54±0.01
20	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.15±0.01	-
21	3-Methylcinnamic acid	-	0.31±0.03
22	Dodecanoic acid	0.11±0.01	2.16±0.49
23	1-Heptadecene	0.91±0.15	6.24±0.97
24	7-Hexadecene, (Z)-	0.11±0.01	-
25	Benzeneacetic acid, 3,4-dihydroxy-	-	7.49±0.50
26	3-Hexadecene, (Z)-	0.11±0.02	0.66±0.09
27	Tetradecanoic acid	0.42±0.09	0.90±0.21
28	1-Octadecene	0.59±0.04	0.82±0.23
29	5-Octadecene, (E)-	0.11±0.02	0.52±0.08
30	9-Octadecyne	-	8.19±0.78
31	2-Pentadecanone, 6,10,14-trimethyl-	0.19±0.01	1.80±0.32
32	Phthalic acid, diisooctyl ester	0.17±0.02	-
33	Hexadecenoic acid, Z-11-	0.27±0.03	-
34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	2.93±0.86
35	n-Hexadecanoic acid	31.10±1.41	12.49±0.69
36	3,5-Dimethoxy-4-hydroxycinnamalde	0.48±0.05	-
37	Trifluoroacetic acid, n-octadec...	-	0.46±0.05
38	Desaspidinol	1.89±0.01	-
39	Phytol	0.04±0.01	3.51±0.38
40	3,5-Dimethoxy-4-hydroxycinnamic acid	0.24±0.01	-
41	9,12-Octadecadienoic acid (Z,Z)-	20.62±0.20	1.47±0.02
42	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	19.90±0.81	16.74±0.78
43	Octadecanoic acid	7.96±0.07	3.71±0.06
44	Octadecanal	0.15±0.01	-
45	9-Octadecenamide, (Z)-	0.39±0.04	-
46	Oxirane, heptadecyl-	0.14±0.02	-
47	Bis(2-ethylhexyl) phthalate	0.31±0.13	-
48	Docosanoic acid	0.26±0.04	-
49	Hexatriacontane	1.37±0.14	
50	Tocopherol	-	13.35±1.46
51	Squalene	0.33±0.02	-

Figures

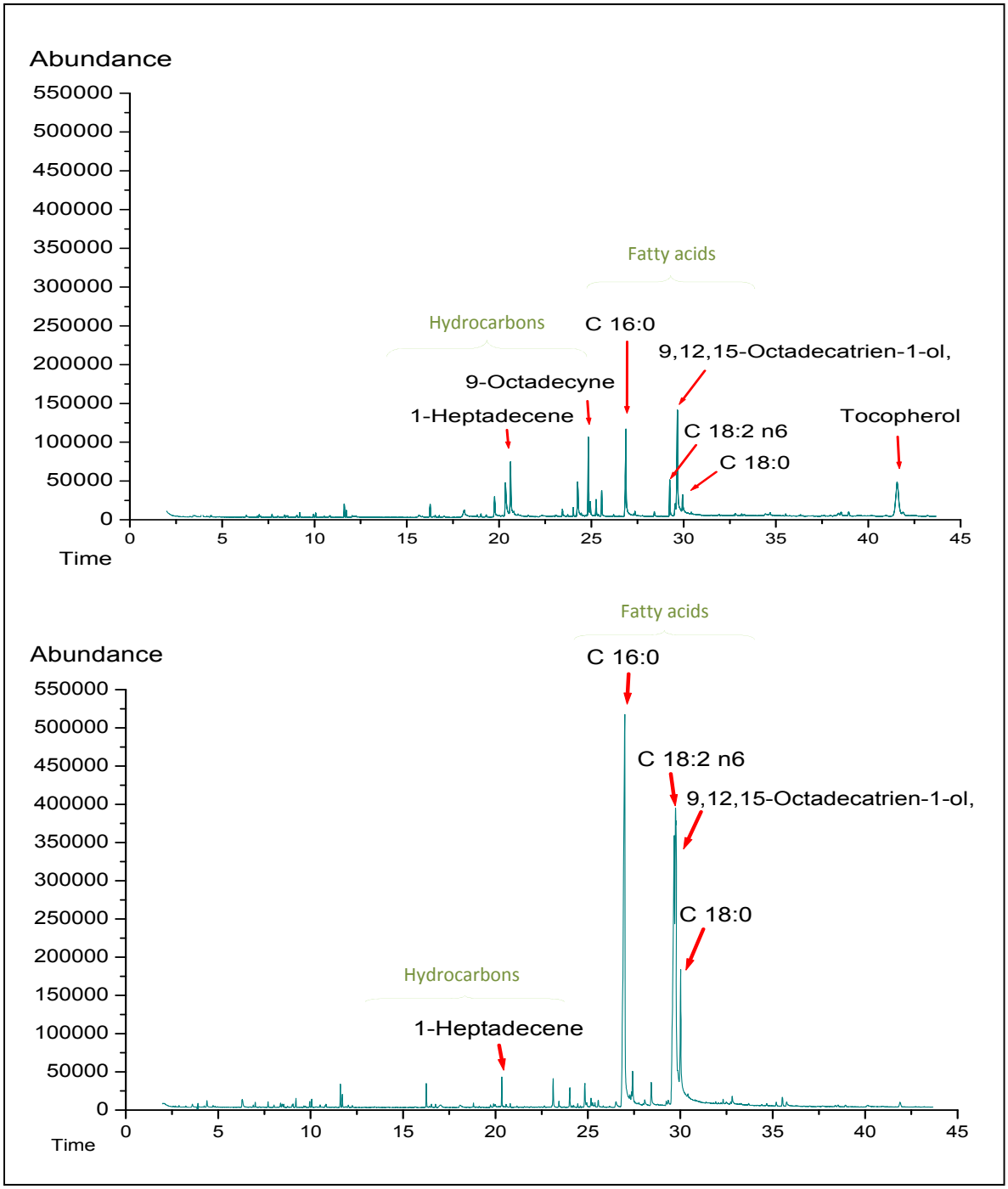

Fig. 1. GC/MS chromatogram of volatile components extracted from leaves of healthy and BLD-affected date palm (*Phoenix dactylifera* L.).

Effect of Certain Insecticides on Some Biochemical Aspects of *Rhynchophorus ferrugineus* (Oliv.)

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Keywords: insecticides, biochemical content, red palm weevil

Abstract

The tested bio agents caused a significant increase in the soluble protein content more than the untreated larvae. In case of the adult males, *Bt.* and *B. bassiana*, caused a non significant elevation in the soluble protein content. However, chlorpyifos-ethyl and imidacloprid caused a significant effect on this parameter. In case of adult females *Bt*, Chlorpyrifos-ethyl and imidacloprid caused a significant increase reaching 167.98, 92.34, 133.64 and 179.58% of the control value, respectively.

***Bt.*, *B. bassiana*, chlorpyifos-ethyl and imidacloprid caused a significant inhibition in acid phosphatase activity of treated larvae. However, *Bt.*, *B. bassiana*, chlorpyifos-ethyl and imidacloprid caused a significant increase in the alkaline phosphates activity.**

***Bt.*, *B. bassiana*, and imidacloprid showed a non-significant alteration of acetylcholinesterase in the treaded larvae and chlorpyifos-ethyl caused significant inhibition compared to the untreated value. As for adult males, *Bt.* caused a significant increase while chlorpyifos-ethyl caused (56.08%) inhibition.**

Considering protease activity the tesed agents, i.e., *Bt.*, *B. bassiana*, chlorpyrifos-ethyl and imidacloprid caused a significant inhibition of larvae. As for adult males the activity was significantly increased. *Bt.*, *B. bassiana*, and imidacloprid caused a significant inhibition in protease of adult females.

The activity of ATP-ases was significantly inhibited by, *Bt.*, *B. bassiana* chlorpyrifos-ethyl and imidacloprid. Total ATP-ases in the treated adult males exhibited a significant increase by *Bt.*, *B. bassiana*, chlorpyrifos-ethyl and imidacloprid, respectively. However the treated adult females, exhibited a general and significant inhibition except for *B. bassiana*.

INTRODUCTION

The *Rhynchophorus* spp. are members of the family *Curculionidae*; the largest natural family of animals. The sub-family *Rhynchophorinas* contains several genera that are closely associated with *Palmae*, *Zingiberaceae*, *Musaceae*, *Amaryllidaceae* and *Gramineae*, all monocotyledons (Kalshoven, 1981). There are more than 40,000 species of weevils in the world and many more to be discovered and named. Practically all feed on plants, most of the larvae burrowing infesting nuts, twigs, and the like (Borror et al. 1989). The adults are usually readily recognized by the shape of the head. The larvae are whitish, usually C-shaped, more or less cylindrical, and usually legless. Many species occurring in the cultivated areas are harmless or not noticeably noxious; other are important pests.

Recently date palm insect pests in general and red palm weevil in particular are widely accepted as being the most destructive and serious insect pest attacking date, coconut and oil palms throughout south and south-east Asia (Wattanapongsiri, 1966). Nowadays, the date palm crop in eastern Arab Countries is under threat. Red palm weevil

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was probably introduced to the Middle East on infested ornamental palm from India or Pakistan. RPW was firstly discovered attacking palm in the Arabian Peninsula especially in the United Arab Emirates in 1985 and progressively spread to Saudi Arabia in 1987 and crossed the red sea into North Africa as the latest record since 1992 in Egypt and finally it appeared in Jordan and Palestine in 1998 (Cox, 1993). Al-Sayji and Shaheen (2005) in Kuwait mentioned that the red palm weevil is the most serious pest of date palm trees. The infestation level is very high in Wafra farms and is spreading to other agricultural areas like Al-Abdali and Al-Jahra. It is found over a wide geographical area in Asia, involving many different agro-ecosystems. The related species is highly polyphagous with a number of known hosts exceeding more than ten different palm species (Murphy and Briscoe, 1999).

Current tactics to manage the weevil in the Gulf and Asia are largely based on applying chemical insecticides. Although there are serious concerns about their effects on the environment. There is now a strong emphasis on the development of integrated pest management based on pheromone traps and biological control rather than on chemical insecticides (Murphy and Briscoe, 1999). However, since RPW resides in self-made tunnels, methods are ineffective. Therefore, self-pathogens have been suggested to be suitable for suppression of this insect pest (Dangar, 1997).

Considering biochemical constituents of red palm weevil, Alarcan et al. (2002) showed a progressive increase of protease activity during larval development of *R. ferrugineus*. Also, Abdel-Razek et al. (2004) revealed an obvious decrease of amino acid composition in the haemolymph of red palm weevil larvae infected by nematode-bacterial complex. Recently, Sharaby and Al-Dosary (2007) proved that the total protcin increased in the cuticle of treated males of red palm weevil with camphene. Acetylcholine esterase and acid phosphatase are not affected. The total lipid increased in the haemolymph.

The present work however was carried out to clarify the efficacy of certain biopesticides as well as synthetic pesticides against certain biochemical constituents of larvae and adults of RPW, *R. ferrugineus*.

MATERIAL AND METHODS

Mortality records and accumulated toxicity were taken for each tested synthetic (chlorpyrifos-ethyl 48% EC and Imidacloprid 25% WP) and biocide (*Beauveria bassiana* and *Bacillus thuringiensis*) insecticides against full grown larvae, adult male and female stages of *R. ferrugineus* (Oliv.) at different days from treatment under laboratory conditions. LC_{50}s were taken for each insecticide and concentrations required to produce LC_{50}s were prepared in water. The sandwich technique was used. Sandwiches were transferred to plastic cups (100 cc) with perforated covers. Full grown larvae as well as adults were introduced into the cups (one/cup), 10 replicates were used for each concentration. Survived individuals after 12 days from treatment were prepared for estimating studied biochemical activities.

Preparation of Samples for Enzyme Assays

The treated full grown larvae, adult males and females of *R. ferrugineus* (Oliv.) were collected, placed in ice containers and homogenized in distilled water using a Teflon homogenizer (Mechianika Precyzyjna Warszawa type MPN-309-Poland) surrounded with a jacket of crushed ice for 3 min. Homogenates were centrifuged at 6000 rpm for 10 min at 5°C (by Beckman GS-6R Centrifuge), and the supernatants were used directly for enzyme assays.

Determination of Total Soluble Proteins

The total soluble proteins were determined in each of the tested larvae and adults by the method of Bradford (1976).

Determination of Acid and Alkaline Phosphatase Activities

Acid phosphatase (AcP) and alkaline phosphatase (AlkP) activities were determined according to the method described by Powell and smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced.

Determination of Acetylcholine Esterase (AChE) Activity

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson et al. (1964) using acetylcholine bromide (AChBr) as substrate at a level of 6×10^{-3} M.

Determination of Protease Activity

The proteolytic activity was determined by the casein digestion method described by Ishaaya et al. (1971).

Determination of Adenosine Tri-Phosphatase (ATP-ase) Activity

The enzymatic activity was determined by the method described by Kobayashi and Akita (1962).

RESULTS AND DISCUSSION

Effect on the Total Soluble Proteins

Data in Table 1 and illustrated in Figure 1 show that the tested agents caused in general a significant increase in the soluble protein content of larvae more than the untreated ones (2.60 mg/g body weight). In terms of figures, the recorded values with *Bt.*, *B. bassiana*, chlorpyrifos-ethyl and imidacloprid in the larval soluble protein were 10.19, 8.40, 7.23 and 12.99 mg/g body weight, respectively. The observed increase in % showed 391.92, 323.08, 278.08 and 499.62% as normal respectively.

In case of the adult males, *Bt.*, *B. bassiana*, caused a non significant elevation in the soluble protein content compared with the normal value (4.50 mg/g body weight). On the other hand, the synthetic insecticides, i.e., chlorpyrifos-ethyl and imidacloprid caused a significant effect on this aspect. In case of the adult females data revealed that *Bacillus thuringiensis*, chlorpyrifos-ethyl and imidacloprid caused a significant increase in such aspect to reach 7.24, 5.76 and 7.74 mg/g body weight compared with the control value of 4.31 mg/g body weight, respectively).

Data clearly indicated that the tested insecticides caused a general increase in the soluble protein content in the treated stages. Such increase is expected since the bacterial insecticide, *Bt.* and the mycoinsecticide, *B. bassiana* are causing an intoxication of the treated insect through secretion of Cry delta endotoxin from *Bt.* and/or fungal toxin. These toxins are proteineous in their structures hence they increase the soluble protein content in the targeted insect (Hoft and Whiteley, 1989; Hori et al., 1994). In more details, it is well documented that the pathogenic fungus or *Bt.* produces a series of proteineous enzymes including amino peptidase, serin dipeptidylpeptidase and other enzymes which may be considered as additional protein content to the affected insect as mentioned by Leger et al. (1987, 1989).

As for the synthetic insecticides, chlorpyrifos-ethyl and imidacloprid, the elevation of the soluble protein content in the treated insect after exposure to such compounds may be due to the mode of action of such substances. In other words, it is well documented that chlorpyrifos-ethyl as organophosphorus compound causes an inhibition of the acetylcholinesterase, hence blocking the hydrolysis of the neurotransmitter, acetylcholine. At the same time the inhibited enzyme and the newly produced enzymes represent an additional source as soluble protein at the time of protein determination in the affected insects. Also, depending on the mode of action of imidacloprid, it is known that such compound is a chlorinated analog of nicotine, and acts on the nicotinic acetylcholine

receptor; the chlorination inhibits degradation by acetylcholineesterase. So that, the acetyl cholinesterase will accumulate at the synaptic region and represents another source of soluble protein content in the targeted insect. In addition, it was found that such compound caused a rise in the glial fibrillary acid protein (GFAP) of the treated animal (Abou-Donia et al., 2008).

Effect on the Activation of Acid and Alkaline Phosphatses

Data in Table 2 and Figure 2 show that the tested agents, i.e., *Bt.*, *B. bassiana*, chloropyrifos-ethyl and imidacloprid caused a general and significant inhibition on acid phosphatase of treated larvae reaching 48.36, 23.97, 22.99 and 3.05 µg phenol/min/mg protein which are far below the control value (71.49 µg phenol/min/mg protein), respectively. In term of figures, the enzyme activity reached 67.65, 33.53, 32.16 and 4.27% as control, respectively.

In addition, the specific activity of alkaline phosphatase (Table 3 and Fig. 3) was increased significantly and *Bt., B. bassiana*, chlorpyrifos-ethyl and imidacloprid caused a significant increase in the alkaline phosphatase activity to reach 8.21, 1.32, 0.91 and 1.15 µg phenol/min/mg protein, respectively.

The specific activity of acid phosphatase exhibited a significant inhibition in the treated larvae with all the tested agents, especially with imidacloprid, followed by chlorpyrifos, *B. bassiana*, and *Bt.* On the other hand, the treated adult males and females did not show any significant alteration in the activity of such enzymes. This may be due to the fact that the pH of the targeted insect haemolymph tends to be alkaline more than acidic which affects in turn the activity of the acid phosphatase as described by Gilmour (1962) and Rocksteir (1978).

For alkaline phosphatase, the specific activity of such enzymes was elevated significantly in the larvae treated with *Bt.*, *B. bassiana*, and imidacloprid. The same trend of elevation was observed in the treated adult stage, either males or females after exposing to *B. bassiana*. In other words, depending on the mode of action of the *Bt.* endotoxin, it was observed that the relation between such toxin and alkaline phosphatase is localizing the binding proteins in the midgut. In such way, it was found that three Cry 1A-binding proteins, viz., aminopeptidase N (APN), the cadherin-like Bt-R1, and membrance-type alkaline phosphatase (m-ALP), were localized, by immunohistochemistry, in sections from the anterior, middle, and posterior regions of the midgut from second instar *M. sexta* larvae. Both APN and m-ALP were distributed predominantly along microvilli in the posterior region and to a lesser extent on the apical tip of microvilli in the anterior and middle regions. Bt-R1 was localized at the base of microvilli in the anterior region, over the entire microvilli in the middle region, and at both the apex and base of microvilli in the posterior region (Chen et al., 2005). Also, it was found that alkaline phosphatase plays a major role as functional receptor of the *Bt.* Cry 11Aa toxin in the affected insects (Fernandez et al., 2006).

In addition, the increase of alkaline phosphatase in the treated insect by *B. bassiana* may be due to the secretion of such enzyme by the pathogenic fungus after its penetration in the insect integument. Such interpretation is supported by the fact that alkaline phosphatase is considered as one of the major biomarkers to distinguish between the different isolates of *B. bassiana*, which are found in the darkling beetle, *Alphitobius diaperinus* (*Coleoptera*: *Tenebrionidae*) (Castrillo and Brooks, 1998).

On the contrary, the inhibition effect of the synthetic insecticide, imidacloprid on the alkaline phosphatase may be due to the effect of such compound on the phosphorylation/nonphosphorylation process in the affected insect as reported by Courjaret and Lapied (2001) who found that the phosphatase activity in *Periplaneta americana* was inhibited through the effect of the neonicotinoid insecticide imidacloprid on the phosphorylation/dephosphorylation of insect neuronal nicotinic acetylcholine receptors (nAChRs).

Effect on the Acetylcholinesterase Activity

Data in Table 4 and Figure 4 show that the treatment with *Bt.*, *B. bassiana*, and imidacloprid caused a non-significant alteration to be 12.07, 16.67 and 15.14 µg AChBr/min/mg protein, respectively. The only exception was related to the effect of chlorpyrifos-ethyl which caused a significant inhibition to reach 8.24 µg AChBr/min/mg protein, representing 54.25% as control.

Data on adult males showed that the bacterial insecticide, *Bt.* caused a significant increase while in enzyme activity chlorpyrifos-ethyl caused a significant inhibition. The activity altered by 185.94 and 56.08% of the control value, respectively. In acse of the adult females such enzyme exhibited a significant increase caused by the two tested biocides, i.e., *Bt.*, *B. bassiana* to reach 17.80 and 14.58 AChBr/min/mg protein. Chlorpyrifos caused a significant inhibition of such enzyme to 6.29 AChBr/min/mg protein 58.19% as the control value.

The elevation of acetylcholinesterase in the treated insect by the biocide agents, may be explained through its release from the neuronal membranes which increase in turn their activity after treatment by such agents. For example, it was found that the mode of acetylcholinesterase release from the neuronal membranes is increased by the action of phosphatidylinositol (PI)-specific phospholipase C of the bacterial insecticide, *Bt.* (Taguchi et al., 1983, 1984).

Inhibition of acetylcholinesterase by chlorpyrifos-ethyl in the treated insect stage is expected since this compound belongs to the organophosphorus insecticides. This group of compounds is considered as anticholinesterase substances as described by Nigg and Knaak (2000) and Abo-Elsaad and Al-Ajlan (2007).

The non significant alteration in the acetylcholinesterase activity with imidacloprid may be attributed to the fact that such compound is a chlorinated analog of nicotine, the compound therefore belongs to the class of chloronicotinyl insecticides which acts on the nicotinic acetylcholine receptor. This means that the chlorination inhibits the degradation by acetylcholinesterase and hence the activity of this enzyme is not affected by the mentioned insecticide (George et al., 2007). In other work, it was found that imidacloprid acts by interfering with the transmission of stimuli in the insect nervous system. Specifically, it causes a blockage in a type of neuronal pathway (nicotinergic) that is more abundant in insects than in warm-blooded animals. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventually death. Such explanation is supported by (Liu-Ming and Casida, 1993).

Effect on the Protease Activity

Data in Table 5 and Figure 5 indicate that all of the tested agents, i.e. *Bt.*, *B. bassiana*, chlorpyrifos-ethyl and imidacloprid caused a significant inhibition of larval protease to reach 13.08, 15.14, 8.55 and 11.86 µg casein/min/mg protein which are far below the untreated value (30.94 µg casein/min/mg protein). The inhibition became 42.28, 49.81, 27.63 and 38.33% as the control value, respectively.

Data on the adult males clearly showed that the protease activity was significantly increased more than the control value (16.62 µg casein/min/mg protein). However, the bacterial insecticide, *Bt.*, the fungus *B. bassiana*, chlorpyrifos-ethyl and imidacloprid caused a significant increase in the protease activity. *Bt.*, *B. bassiana*, and imidacloprid caused a significant inhibition to reach 17.87, 27.86 and 19.20 µg casein/min/mg protein, compared with 34.40 µg casein/min/mg protein, respectively.

When the selected insecticides tested against the larvae, adult males and females of the RPW, significant alterations (+,-) were observed in the specific activity of tested enzyme. According to the literature cited, it is well documented that a progressive increase of protease activity has been found in *R. ferrugineus* during the larval development (Alarcon et al., 2002). From another viewpoint, when an insect pest is exposed to a bioagents such as *Bt.*, it is expected that such treatment results in an inhibition of the activity of such enzyme in the susceptible strains and conversely in the

resistant one. However, other investigators revealed that toxin of such bacterial insecticide caused changes in the protease activity in the targeted insect pest as mentioned by Loseva et al. (2002).

On the other hand, several researchers reported that the pathogenic fungus such as *B. bassiana* causes an increase in such enzyme in the targeted insect through its capacity to secrete a wide range of protease enzymes such as serine endoprotease and cystein cndoprotease which help the pathogenic fungus to penetrate the integument of the targeted insect as described by Leger et al. (1989) and Goettel et al. (1989). Generally, the non-uniform response of the protease activity after exposing the targeted larvae and/or adult stages may be due to the heterogeneous application of the tested agents which differed between the dipping technique in the fungal preparation and/or feeding technique for treatment of the tested stages by synthetic insecticide and the bioagent, *Bt.* Such differences may cause the non clear response of such enzyme activity towards the mentioned tested agents.

Effect on the Activity of Total ATP-ases

Data in Table 6 and Figure 6 showed that the ATP-ases enzymatic system was significantly inhibited by *Bt.*, *B. bassiana*, chlorpyrifos-ethyl and imidacloprid to reach 0.12, 0.14, 0.13 and 0.10 µg Pi/min/mg protein. The control value was 0.57 µg Pi/min/mg protein. The inhibition percentage reached 21.05, 24.56, 22.81 and 17.54% of the control value, respectively.

On the contrary, the total ATP-ases in the treated adult males exhibited a significant increase compared to the normal value (0.17 µg Pi/min/mg protein). In terms of figures, the ATP-ases reached 1.50, 0.30, 0.25 and 0.20 µg Pi/min/mg protein with the same insecticides, showing 882.35, 176.47, 147.06 and 117.65% as the control value, respectively.

In case of total ATP-ases in the treated adult females, the specific activity of such enzymatic system exhibited a general and significant inhibition as affected by the tested agents, except for *B. bassiana* which caused a significant increase in this respect. Accordingly *Bt.*, chlorpyrifos-ethyl and imidacloprid caused alteration reaching 67.74, 132.26, 96.77 and 64.52% as control respectively.

Generally, the inhibition of the total ATP-ases is expected since that such enzyme works for a wide range of biochemical reactions in different metabolic pathways. For example, at the neuronal membranes, it is documented that the neurotransmission process through Na^+/K^+ pump along the nerve axons is based on a consumption of energy, a process that requires the availability of adenosine triphosphate (ATP) molecules and ATP-ases to liberate energy from the cleavage the phosphate bond of the ATP molecules to produce adenosine diphosphate (ADP) and inorganic phosphate (Pi). This mechanism is actually controlled by a wide variety of adenosine triphosphatases enzymes which include Ca^{2+} ATP-ases, Mg^{2+} ATP-ases and Na^+/K^+ ATP-ases. At the same time, it has been documented for many years that ATP-ases are associated with muscular activity, mitochondrial processes as well as with the activities of nerve cell membranes. Thus, different ATP-ases are associated with the activities of nerve cells depending on the presence of sodium and potassium ions, calcium ions and magnesium ions. So that, ATP-ases are not necessarily all located in the axonic memberance itself, but some could be associated with the mitochondria, which provide energy for the pump or affecting the release of the neurotransmitter from the presynaptic vesicle.

According to such facts, several researchers reported that the cytotoxic effect of the toxic substances, such as pesticides on the activities of total ATP-ases may be due to the inhibitory effect of the tested compound on the phosphatases enzymes which affect in turn the activity of ATP-ases as described by Siddiqui et al. (1993). Moreover, since the mitochondria of the neurons or the non targeted cells are the main site of the biosynthesis of the energy molecules (ATP), so that, there is a high possibility that the mitochondria could be malformed as a side effect to the tested compounds which may lead to decrease the total amount of ATP which results in turn in a reduction in the activitity of ATP-ases

(Muscatello et al., 1975).

A similar finding was reported when bioagents such as *Bt.* were tested against various insect pest species. For example, English and Cantley (1985) found that the delta endotoxin, a 68 kilodalton protein isolated from *Bt.* spp. *Kurstaki*, decreased in cytosolic pH in a cell line (CHE) originating from *Manduca sexta* embryonic tissue and caused inhibition of K^+-sensitive-ATP-ases in the plasma membranes isolated from these cells.

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Tables

Table 1. Effect of the tested biocides and conventional insecticides on the soluble protein content in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	Soluble protein content (mg/g bw)	%	Soluble protein content (mg/g bw)	%	Soluble protein content (mg/g bw)	%
Bacillus thuringiensis	10.191±0.11***	391.92	5.01±0.09***	111.33	7.24±0.11*	167.98
Beauveria bassiana	8.400±0.12***	323.08	5.52±0.12*	122.66	3.98±0.10	92.34
Chlorpyrifos-ethyl	7.230±0.11***	278.08	6.54±0.11	145.33	5.76±0.11*	133.64
Imidacloprid	12.990±0.13***	499.62	7.51±0.11	166.88	7.74±0.11*	179.58
Control	2.600±0.12	100.00	4.50±0.11	100.00	4.31±0.13	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Table 2. Effect of the tested biocides and conventional insecticides on the specific activity of acid phosphatase enzyme in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	Acid phosphatase (µg phenol/min/mg protein)	%	Acid phosphatase (µg phenol/min/mg protein)	%	Acid phosphatase (µg phenol/min/mg protein)	%
Bacillus thuringiensis	48.36±0.11***	67.65	17.33±1.52	106.58	25.00±1.20	110.23
Beauveria bassiana	23.97±0.15***	33.53	23.30±0.18*	143.30	22.17±1.00	97.75
Chlorpyrifos-ethyl	22.99±0.13***	32.16	14.87±0.13	91.45	26.55±1.43	117.06
Imidacloprid	3.05±0.11***	4.27	19.74±0.12	121.40	26.99±1.30	119.00
Control	71.49±0.59	100.00	16.26±0.03	100.00	22.68±1.17	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Table 3. Effect of the tested biocides and conventional insecticides on the specific activity of alkaline phosphatase in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	Alkaline phosphatase (µg phenol/min/mg protein)	%	Alkaline phosphatase (µg phenol/min/mg protein)	%	Alkaline phosphatase (µg phenol/min/mg protein)	%
Bacillus thuringiensis	8.21±0.01***	1282.81	0.79±0.08***	15.39	0.93±0.01	73.23
Beauveria bassiana	1.32±0.01*	206.25	19.12±0.26***	385.48	4.74±0.41***	373.23
Chlorpyrifos-ethyl	0.91±0.01	142.19	3.42±0.12	68.95	1.63±0.14	128.35
Imidacloprid	1.15±0.01*	179.69	2.46±0.11	49.60	0.51±0.01***	40.16
Control	0.64±0.01	100.00	4.96±0.11	100.00	1.27±0.01	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Table 4. Effect of the tested biocides and conventional insecticides on the specific activity of acetylcholinesterase in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	AChEase (µg ChBr/min/mg protein)	%	AChEase (µg ChBr/min/mg protein)	%	AChEase (µg ChBr/min/mg protein)	%
Bacillus thuringiensis	12.07±1.11	79.46	21.55±5.14*	185.94	17.80±1.01**	164.66
Beauveria bassiana	16.67±0.23	109.74	12.62±0.03	108.89	14.58±0.18*	134.88
Chlorpyrifos-ethyl	8.24±0.82**	54.25	6.50±0.43**	56.08	6.29±0.91**	58.19
Imidacloprid	15.14±3.74	99.67	9.25±1.85	79.81	8.47±1.20	78.35
Control	15.19±2.50	100.00	11.59±0.14	100.00	10.81±0.15	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Table 5. Effect of the tested biocides and conventional insecticides on the specific activity of protease in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	Protease (µg casein/min/mg protein)	%	Protease (µg casein /min/mg protein)	%	Protease (µg casein /min/mg protein)	%
Bacillus thuringiensis	13.08±0.20***	42.28	135.07±10.81***	812.70	17.87±0.28**	51.95
Beauveria bassiana	15.41±0.48***	49.81	28.81±0.65*	173.35	27.86±0.76*	80.99
Chlorpyrifos-ethyl	8.55±0.39**	27.63	48.75±0.58**	293.32	38.43±0.53	111.72
Imidacloprid	11.86±0.34***	38.33	36.50±0.39**	219.61	19.20±0.66***	55.81
Control	30.94±0.37	100.00	16.62±0.41	100.00	34.4±0.59	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Table 6. Effect of the tested biocides and conventional insecticides on the specific activity of total ATP-ases in the full grown larvae, adult males and females of the RPW after 12 days from treatment.

Tested agents	Larvae		Adult male		Adult female	
	Total ATP-ases (µg Pi/min/mg protein)	%	Total ATP-ases (µg Pi/min/mg protein)	%	Total ATP-ases (µg Pi/min/mg protein)	%
Bacillus thuringiensis	0.12±0.01***	21.05	1.50±0.12***	882.35	0.21±0.01**	67.74
Beauveria bassiana	0.14±0.01***	24.56	0.30±0.01**	176.47	0.41±0.01*	132.26
Chlorpyrifos-ethyl	0.13±0.01***	22.81	0.25±0.01*	147.06	0.30±0.01	96.77
Imidacloprid	0.10±0.01***	17.54	0.20±0.01	117.65	0.20±0.01**	64.52
Control	0.57±0.02	100.00	0.17±0.01	100.00	0.31±0.02	100.00

Each value represents the average of three replicates ± standard deviation. Comparing to the control values, ***: highly significant (p<0.001), **: moderately significant (p<0.01), *: significant (p<0.05) (student t-test).

Figures

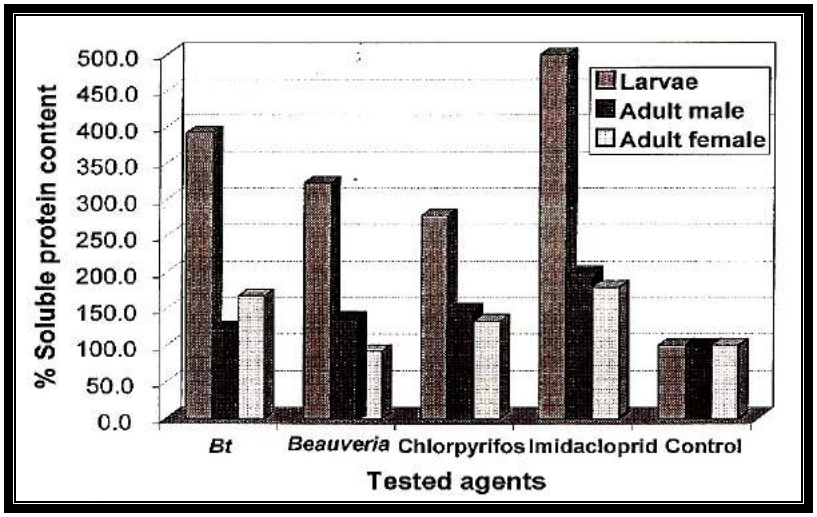

Fig. 1. Effect of the tested biocides and insecticides on the percentage of soluble protein content in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

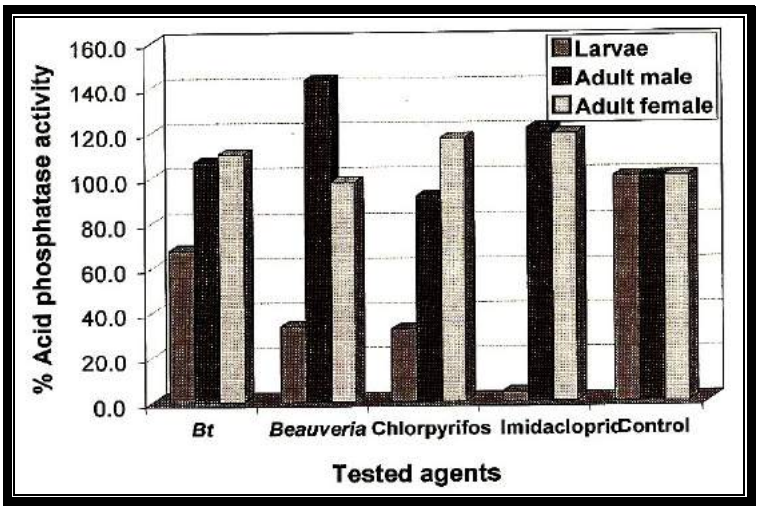

Fig. 2. Effect of the tested biocides and insecticides on the activity percentage of acid phosphatase in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

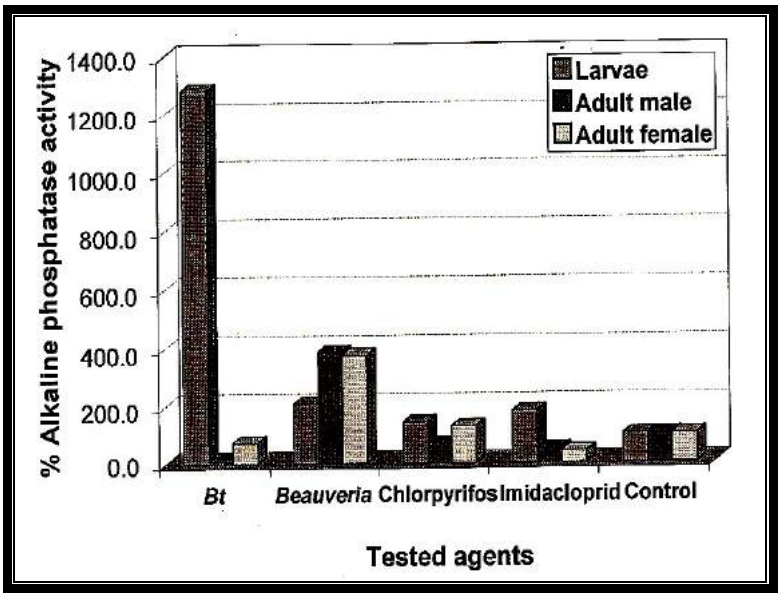

Fig. 3. Effect of the tested biocides and insecticides on the activity percentage of acid phosphatase in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

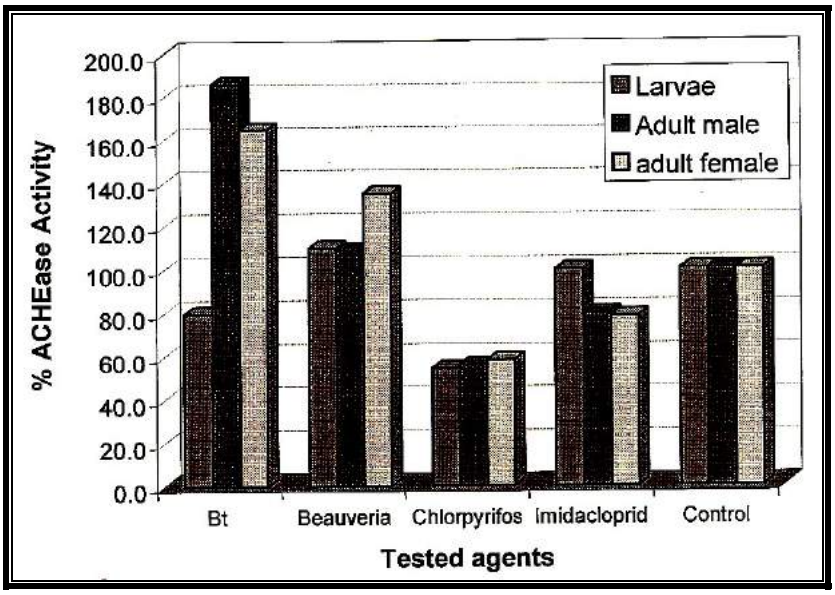

Fig. 4. Effect of the tested biocides and insecticides on the activity percentage of acetylcholinesterase in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

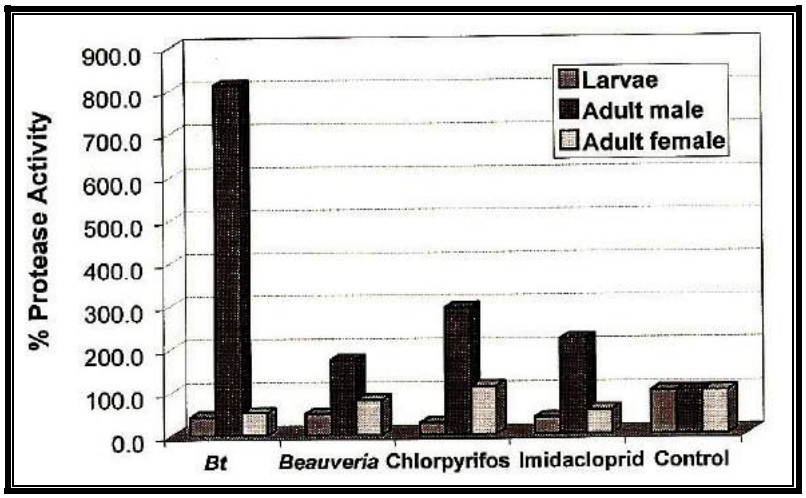

Fig. 5. Effect of the tested biocides and insecticides on the activity percentage of protease in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

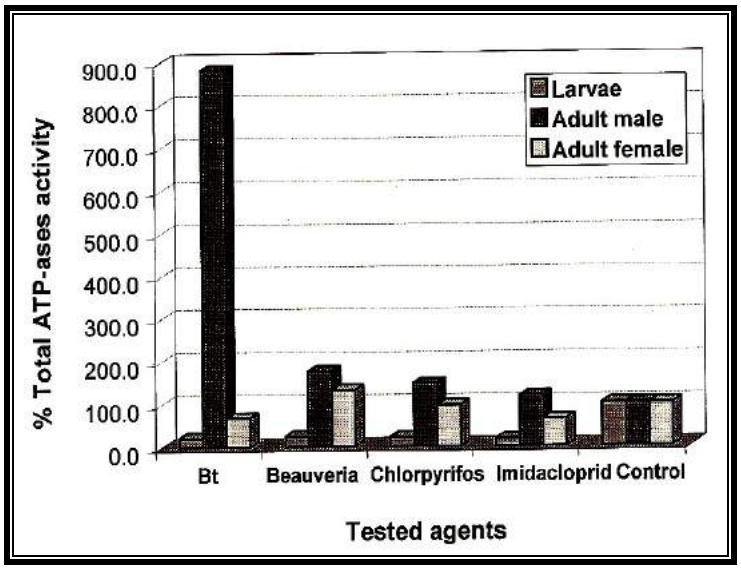

Fig. 6. Effect of the tested biocides and insecticides on the activity percentage of total ATP-ases in the full grown larvae, adult males and adult females of the RPW after 12 days from treatment.

Factors Influencing the Effectiveness of Certain Novel Insecticides against Red Palm Weevil

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Keywords: synthetic insecticides, *Beauveria bassiana*, *Bt.* toxin, *Rhynchophorus ferrugineus*

Abstract

The present work was carried out at the Middle East Regional Laboratory of red palm weevil, El-Kassasin, Ismalia Governorate to evaluate the efficiency of four synthetic insecticides (i.e., Chlorpyifos, Imidacloprid, Chlothianidine 50% WG and 16% SG) compared with two bio-insecticides (i.e., *Beauveria bassiana* and *Bt.* toxin) against full grown larvae and adults of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) under laboratory conditions. The obtained results showed that the rate of toxicity varied tremendously according to the nature of the applied toxicant, used concentration, time elapsed after exposure and the treated stage. Also, as the time after application progressed there was an increase in the rate of accumulated mortality. The full grown larvae proved to be more susceptible when compared with the adult stage. Imidacloprid seemed to be the most effective toxicant against full grown larvae when compared with the other tested synthetic insecticides. In case of adult stage, Chlorpyrifos proved the most influential in this respect. Regarding the two tested biocides, the LC_{50}s of *Beauveria bassiana* after 15 days showed 1.08×10^5 and 2.2×10^5 spores/ml against full grown larvae and adult stage, respectively. In case of *Bacillus thuringiensis* it gave 798.73 and 7600 ppm, against full grown larvae and adult stage, respectively.

INTRODUCTION

The date palm and the date fruits are hosts for many insects and diseases which are seriously enough to inflict heavy losses if left uncontrolled. Under traditional date palm culture the growers were helpless and in some cases they were unable to identify the causal organism. Several examples can be cited in that respect, the worst example being the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). The cause of the high rate of spread of this pest is human intervention, by transporting infested young or adult date palm trees and offshoots from infested to clean areas. Because of the concealed nature of red palm weevil larvae (the main stage causing the injury), effective methods to control this pest have been difficult to develop. During the last two decades all efforts to control *R. ferrugineus* (Olivier) in the Arab Countries, focused on the use of traditional insecticides, modified cultural practices and recently, pheromone traps (Abraham et al., 1998).

Recently there is much intent to reduce the contusive use of chemical insecticides and to prevent development of insect resistance. The most promising issues to achieve this goal including rotation between traditional insecticides and new control agents. So, the present work was carried out to study the efficacy of certain bio-pesticides as well as synthetic pesticides against larvae and adults of red palm weevil, *R. ferrugineus* (Olivier).

MATERIAL AND METHODS

Adults of red palm weevil *R. ferrugineus* (Olivier) were collected from the fields

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cultivated with date palm trees located at El-Kassassin district (of El-Ohda village), Sharkia Governorate. Newly hatched larvae were reared on artificial media according to Rahalkar et al. (1985).

Synthetic insecticides tested were Chlorpyifos (Chlorzan 48% EC), Imidacloprid (Pestidor 25% WP) and Chlothianidine (S-1165 50% WG and 16% SG). The tested biocides were a fungal preparation of *Beauveria bassiana* (1×10^9 spores/ml) and *Bt.* toxin (Agerin 6.5%WP). The entomopathogenic fungi of *B. bassiana* used in the experiments were originally isolated from red palm weevil *R. ferrugineus* (Olivier) in Ismailia Governorate in Egypt and prepared according to Sewify and Fouad (2006).

Serial concentrations for each material were prepared in water. The Sandwich technique was used for insect treatment in case of synthetic insecticides, but dipping sugarcane stalks for 15-30 s in the prepared solutions was conducted with the two tested bio-insecticides. Healthy full grown larvae and adults were carefully selected and introduced into plastic cups (100 cc.), each individual per cup. Ten replicates were used for each concentration. All treatments as well as an untreated check were kept at constant conditions 29±1°C, 70% RH and 14-h photoperiod. Mortality counts were recorded at 3, 6, 9, 12 and 15 days from application. The observed mortality was corrected when necessary by using Abbott's formula (1925). Toxicity lines were established where the probit units of percent mortalities were plotted against a logarithmic scale of concentration (Finney, 1971) to obtain LC_{50}s, LC_{90}s and slope values.

RESULTS AND DISCUSSION

Effect of Synthetic Insecticides on Red Palm Weevil

Data in Table 1 show the LC_{50}s and relative potencies of the tested insecticides against both full grown larvae and adult stage of red palm weevil, *R. ferrugineus* (Olivier). The LC values showed that full grown larvae were more susceptible to the tested compounds than the adult stage. Also, the rate of toxicity varied tremendously according to the type of toxicant, concentration used and time elapsed after exposure. Based on the comparative potencies for the full grown larvae data indicated that imidacloprid (Pestidor 25% WP) proved to be the most superior insecticide followed by chlorpyrifos (Cholrzane 48% EC), chlothianidine (S-1165 16% SG) and chlothianidine (S-1165 50% WG), respectively. The relative potency based on the most inferior toxicant (chlothianidine (S-1165 50% WG)=1.00) showed 1.47-, 1.30- and 1.06-fold for imidacloprid (Pestidor 25% WP), chlopyrifos (Chlorzane 48% EC) and chlothianidine (S-1165 16% SG), respectively. In case of the adult stage Chlorzane 48% EC seemed to be the most effective toxicant followed by chlothianidine (S-1165 16% SG), chlothianidine (S-1165 50% WG) and imidacloprid (Pestidor 25% WP), respectively. The relative potency based on the most inferior toxicant (imidacloprid (Pestidor 25% WP)=1.00) gave 1.63-, 1.29- and 1.04-fold for chlorpyrifos (Chlorzane 48% EC), chlothianidine (S-1165 16% SG) and chlothianidine (S-1165 50% WG), respectively.

Imidacloprid (Pestidor 25% WP) seemed to be the most potent toxicant against full grown larvae. However, Chlorpyrifos (Chlorzane 48% EC) showed the superior toxicant against the adult stage. The obtained results emphasized the higher susceptibility of the larval stage when compared with the adult which was reported by Barranco et al. (1998), Ajlan et al. (2002) and Beevi et al. (2004).

Effect of Biocides on Red Palm Weevil

1. Effect of *Beauveria bassiana*. Data in Table 2 showed the influential role of exposed stage and time after treatment in determining the rate of toxicity of entomopathogenic fungus. The obtained LC_{50}s noticeably decreased by the advancement of time after treatment showing 3.27×10^7, 2.00×10^6, 5.54×10^5 and 1.08×10^5 spores/ml after 6, 9, 12 and 15 days after application. Accordingly, the relative potencies based on 6 days after treatment gave 16.35-, 59.03- and 342.59-fold after 9, 12 and 15 days after treatment, respectively. In case of the adult stage, the LC_{50} values were 5.42×10^7, 3.40×10^6,

1.11×10^6 and 2.2×10^5 spores/ml after 6, 9, 12 and 15 days after application. The relative potencies based on 6 days after treatment (=1.00) showed 15.85-, 48.83- and 246.36-fold after the above-mentioned intervals, respectively.

In this respect different investigators isolated entomopathogenic fungi *B. bassiana* and *Metarhizium anisopliae* from *R. ferrugineus* such as Ghazavi and Avand-Faghih (2002), Ghazavi et al. (2002) and Shaiju-Simon et al. (2003). Other investigators studied the activity of such fungi in controlling red palm weevil such as Saleh et al. (2004) and Gindin et al. (2006). Also, Sewify and Fouad (2006) suggested that, control of *R. ferrugineus* (Olivier) in infested palm trees is highly possible with a combination of fungus *B. bassiana* with essential mint oil and antimoulting consulto. However, El-Sufty et al. (2007) mentioned that the young larvae of red palm weevil were more susceptible than the old ones by using entomopathogenic fungus *B. bassiana.*

2. Effect of *Bacillus thuringiensis* (Agrin 6.5% WP). Data in Table 3 indicate that the toxicity of bioagent *Bt.* (Agerin 6.5% WP) against full grown larvae and adults of red palm weevil varied considerably based on period elapsed after treatment and the exposed stage. It is worth to note that the accumulated mortality was progressively increased by the advancement of days after application especially with the higher concentration. The adult stage showed to be more tolerant compared with the full grown larvae. The LC_{50}s of *Bt.* against full grown larvae showed 1468.8, 1198.9 and 798.73 ppm after 9, 12 and 15 days from application. Taking the 9^{th} day after treatment as a base line for calculation (=1.00), the relative potency revealed 1.23- and 1.84-fold after 12 and 15 days from application. In case of the adult stage, the LC_{50}s gave >10010, 8000 and 7600 ppm, after 9, 12 and 15 days from treatment. Accordingly, the relative potency based on the 9^{th} day after treatment exhibited 1.25- and 1.32-fold after 12 and 15 days from application.

These data agree with Salama et al. (2004), Saleh et al. (2004) and Sewify and Fouad (2006) and enhance the possibility of using such bio-control agents as a main element for an IPM programme of red palm weevil.

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Tables

Table 1. LC values and relative potencies of tested synthetic insecticides against full grown larvae and adult stage of red palm weevil, *R. ferrugineus* (Olivier) after 12 days from application.

Stage	Insecticides	LC_{50} (ppm)	LC_{90} (ppm)	Slope (b)	Relative potency
Full grown larvae	Chlorzane 48% EC	499.99	712.87	8.420	1.30
	S-1165 50% WG	651.90	868.70	10.100	1.00
	S-1165 16% SG	612.60	831.80	4.600	1.06
	Pestidor 25 % WP	443.80	840.90	4.599	1.47
Adult stage	Chlorzane 48% EC	612.58	907.13	7.480	1.63
	S-1165 50% WG	962.80	1390.00	1.760	1.04
	S-1165 16% SG	775.30	1020.00	7.860	1.29
	Pestidor 25 % WP	997.30	1125.00	3.397	1.00

Table 2. LC_{50}s and relative potencies of entomopathogenic fungus (*B. bassiana*) against full grown instar larvae and adult stage of red palm weevil, *R. ferrugineus* (Olivier)at different days from treatments under laboratory conditions.

Treated stage	Days after treatments	LC_{50} (spores/ml)	LC_{90} (spores/ml)	Slope (b)	Relative potency
Full grown larvae	6	3.27×10^{7}	$1.00\times10^{8}>$	0.676	1.00
	9	2.00×10^{6}	3.14×10^{7}	1.070	16.35
	12	5.54×10^{5}	1.12×10^{7}	0.962	59.03
	15	1.08×10^{5}	8.00×10^{6}	0.763	342.59
Adult stage	6	5.42×10^{7}	$1.00\times10^{8}>$	0.629	1.00
	9	3.40×10^{6}	$1.00\times10^{8}>$	0.767	15.85
	12	1.11×10^{6}	1.88×10^{7}	1.040	48.83
	15	2.20×10^{5}	1.00×10^{7}	0.854	246.36

Table 3. LC_{50}s and relative potencies of *B. thuringiensis* product (Agerin WP 6.5%) against full grown larvae and adult stage of red palm weevil, *R. ferrugineus* (Olivier) at different days from treatments under laboratory conditions.

Treated stage	Days after treatments	LC_{50} (spores/ml)	LC_{90} (spores/ml)	Slope (b)	Relative potency
Full grown larvae	9	1468.80	8400	1.03	1.00
	12	1198.90	>10000	1.14	1.23
	15	798.73	>10000	1.25	1.84
Adult stage	9	10010.00	>10000	3.26	1.00
	12	8000.00	>10000	4.22	1.25
	15	7600.00	>10000	3.14	1.32

The Future Visions and the Influence of Climatic Factors on the Spatial Distribution of Dubas Bug *Ommatissus lybicus* (Debergevin) on Date Palm Trees in Iraq

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Keywords: date palm hopper, Al-Anbar, dust storm, Euphrates, hit, new spread

Abstract

Date palm trees and fruits are subjected to infestation by many serous pests causing a big decline in date production and quality. Infestation distribution and severity are varied according to kind of infestation and species of the pest along with the governing environmental factors. The dubas bug *Ommatissus lybicus* (Debergevin) is considered as a key pest attacking date palm trees mostly in the central region of Iraq. This pest was almost absent in Al-Anbar Province during the past years, however, field survey conducted in different regions of this province during spring and fall seasons of the year 2007 indicated the presence of dubas bug on date palm trees in some orchards toward the east of Hit city. The second generation of the pest was the most damaging in terms of affected trees and accumulation of honey dew. Scattered infestations were also recorded in some orchards west of Hit city toward Al-Qaim at the upper Euphrates regions. Since the spread of this pest occurred in a discontinuous manner with no correlation with closely infested Provinces, this study was initiated to explore the limiting factors influencing the spatial distribution of dubas bug in Iraq with special emphasis on Al-Anbar Province. Results indicated that the mean insects density was 40 nymphs/leaflet recorded during hatching time, at the last week of April and early May of 2008 , reduced to less than 5 nymphs/leaflet during the third week of May of the same year .This reduction was attributed to the severe winter of 2008 in which the minimum temperature dropped below zero in Al-Anbar Province for several days causing the death of date palm leaves in many orchards and resulted in harmful effects on dubas eggs inserted in these leaves. The continuous occurrence of dust storms was another factor which had harmful effects on moving stages. The reduction of dubas infestation to non-damaging levels was continued during spring and fall generations of 2008 and 2009 as a result of the indicated climatic factors. Insects were mainly found on offshoots near the ground with a density ranging between 1-15 individuals/tree. Results have also indicated that the arrival or transmission of the pest to some regions of Al-Anbar Province happened in a way other than the normal distribution due to the limiting climatic factors, therefore, this pest may not persist in the region for a long time. The population might be reduced to non-damaging levels or even disappear during the coming few years. A reliable monitoring system is essentially needed in order to keep the pest under control within a comprehensive IPM program against date palm pests in the country.

INTRODUCTION

Date palms are considered as the most important fruit trees in Iraq. There are more than 650 described cultivars along with too many others still non described in the country (Al-Ansari and Saleh, 2005; El-Haideri and Al-Hafeedh, 1986; Hussain and Hureab, 2004; Zaid, 2002). Date palm trees and fruits are subjected to infestation by many pests

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causing a serious decline in date production and quality. Infestation distribution and severity are varied depending on the kind and pest species and the governing environmental factors. The main pests include: the lesser date moth (*Humera*), *Batrachedra ammydraula* Meydrick, the borer complex such as the longhorn stem borer, *Jebusaea hammerschmidti* Reiche, the bunch stalk borer, *Oryctes elegans* Preil., the frond borer, *Phonabate frontalis* Fahraeus, the date palm hopper (dubas bug), *Ommatisus lybicus* Debergevin, the dust mite *Oligonychus afrasiaticus* (McGregor). Other pests such as scale insects and fruit insects are of minor importance and are expected to become a major pests at any time due to poor monitoring and management systems (Ali, 2007a; Ba-Angood et al., 2009; El-Haidery and Al-Hafeedh, 1986). The dubas bug is considered the most important pest attacking date palm trees mainly in the middle region of Iraq up to Dyala Province (Ali, 2007a; Al-Khafegi, 1995; Asche and Wilson, 1989; Jasim, 2007). This pest was almost absent in Al-Anbar Province till 2007 when localized infestations were observed toward the east of Hit city which is located in this Province (Ali, 2007b). The distribution of this pest in general is heavily influenced by climatic factors (Ali, 2007a; Ali et al., 2009; Al-Khafagi et al., 1995; Jasim, 2007). Infestation is varied between orchards and from one year to another depending on cultural practices and governing environment factors (Ali, 2007a; Al-Khafagi, 1995; Jasim, 2007). This pest is controlled mainly by using non-specific chemical pesticides applied as an aerial or ground spray (Al-Jboory et al., 2001; Hama et al., 2002). Previous experiences indicated that chemical insecticides are not sufficient for controlling date palm pests and reducing their damage significantly. Furthermore, the use of aerial and ground application tools contributed in complicating the problem in terms of environmental pollution and human hazards along with the influences on non-targeted organisms. Therefore, the adoption and application of an IPM project for controlling date palm pests is an essential need. The use of natural enemies such as predators, parasitoids and insect pathogens proven to be effective control agents and can be considered in the future management practices against these pests. Other non-poisonous means should be considered as a first choice along with a good management of agricultural practices. Specific insecticides can only be used in an urgent case in order to conserve natural enemies and minimize environmental hazards (Hssan et al., 2003; Jasim, 2007; Saleh et al., 2003). Since the date production and other components of date palm trees contribute to a big share of farmer income in middle and southern parts of the country, therefore, there is a need for improvement of date palm orchards and the date production system in general. One of the principle approaches to be applied is a reliable system for date palm pests management including dubas bug. Since the environmental factors showed an obvious effect on geographical distribution of this pest in Iraq (Ali, 2007a), and because the sudden appearance of the pest in Al-Anbar Province with no continuous spread to nearby infested provinces, therefore, this experiment was initiated to investigate the possible means of transportation of dubas to the new region and to identify the limiting factors that influence the spread of this pest.

MATERIALS AND METHODS

The Al-Anbar province was considered as a case study for the purpose of this experiment. Samples were taken along the Euphrates river basin starting from Alfaluga city up to Al-Qaim city close to the Syrian border during spring and fall seasons of 2007 and 2008. Three orchards were chosen as a representative for each intended sampling region and three trees were assigned randomly to represent each orchard. Two leaves and fifteen leaflets representing the base, middle and the terminal parts of each leaf were taken randomly from each tree. Sampling was done for insects and signs of infestations. The following proposed parameters were used for indicating the level of infestation in the study regions (Ali, 2009): 1 = insects are present with an average equal to less than one/orchard; 2 = presence of 1-10 insects/tree in any orchard in the region; 3 = infestation symptoms are clearly identified through the presence of moving stages and molting skins along with the presence of some spots of honey dew on parts of the leaves; 4 = insect damage is obvious through the presence of moving stage and accumulation of honey dew

on leaves and other plants grown under date palm trees.

Since this pest was observed for the first time close to Hit city in Al-Anbar Province during 2007 (Ali, 2007b) a comprehensive study was initiated in this region to explore the source of infestation and the influence of climatic factors on the future of dubas bug in the regions. Three heavily infested orchards were taken on each left and right side of the Euphrates river about 3 km towards the east of Hit city. Samplings were taken during spring and fall seasons of 2008 and 2009. Two leaves representing the southern and northern directions were taken from each of three trees located close to the river, middle and terminal parts of the orchards. Five leaflets were taken from the base and middle and terminal parts of each leaf. Each leaflet was examined to record the presence of insect stages and any other signs of infestation or damages. Sampling was taken during the third week of April and the third week of May in spring and one sample was taken during the first week of October in fall of 2008 and 2009. In order to determine the limiting climatic factor for dubas bug spread, data on the occurrence of rain fall and dust storms along with other factors were also recorded during the presence of moving stages in spring 2008 and in april alone in 2009 which coincided with the presence of early nymphal stages of dubas in the region.

RESULTS AND DISCUSSION

Previous studies indicated that dubas bug is almost present in the central Euphrates regions. Heavy infestation is extended toward Baghdad, Wasit and Dyala provinces while decreased levels are usually found west of Baghdad regions (Fig. 1) (Ali, 2007a; Jasim, 2007). Results of this study indicated no continuous distribution of the pest from Baghdad toward Al-Anbar Province. The pest was found in scattered forms on both sides of the Euphrates river with different levels of infestations. However, the most damaging level was found in orchards at the two sides of Euphrates river about 3 km toward the east of Hit city which was assigned as parameter 4 (Table 1). Field survey indicated that the mean number of nymphs ranged between 40 to 50 individuals/leaf recorded in the last week of April and early May 2008 reduced to about 1-5 nymphs/leaf during the third week of the same month of the same year. Studies conducted in an infested orchard near Hit city have showed that population of dubas on trees at the left side of the river was still too low for both spring and fall generations during 2008 and 2009. Insect stages were almost absent from the main high date palm trees. However, noticeable individuals were found on small trees (ground offshoots) growing under the mother tree. The number of insects varied between 1 to 10 individuals/tree depending on location and density of trees in the orchards (Table 2). Results have also indicated that dubas density per tree on the right side of the river followed the same trend of the left side with some apparent differences. Insects were also mainly found on small trees with density ranging between 1-15 individuals per tree depending on season, age and location of trees in the orchards and that was assigned the parameters 2-3 (Table 3). Field survey indicated that farmers at the left side followed the directions of plant protection specialists for chemical insecticide application by ground means during the spring generation of 2008. While most of the orchard owners at the right side did not apply any chemical treatment. Therefore, the reduction of insect population and infestation levels at both sides of the river in the region could be attributed to other factors such as cultural practices and climatic factors including rain, wind and dust storms. The decreased levels of infestation could be attributed to the severe winter of 2008 in which the minimum temperature dropped below zero in Al-Anbar Province for several days causing the death of date palm leaves in many orchards and which resulted in harmful effects on dubas eggs inserted in these leaves. The shortages of rainfall contributed to the increasing levels of dryness and that would help the development of rising dust and the formation of dust storms. Result showed that the frequency of dust storms was clearly high during the active period of dubas in spring 2008. The number of dust storms was 10, 13, 12 recorded in April, May and June respectively (Table 4). However, it had been reported that there were 11 dust storms in April 2009 alone. Storm density may have varied during the same

day with continuous presence of light dust in most days during the month. Previous studies indicated that eggs hatching usually starts in late March or early April (Al-Hafeedh, 1988; Al-Khafagi et al., 1995; Jasim, 2007), therefore the occurrence and frequency of dust storm happened during the same period of the presence of young moving stages of the dubas. The presence of the dust could be the most detrimental factor causing the death for the pest and could be the main element responsible for the elimination of this pest from the tall date palm trees. The insects tried to move away towards the ground in order to find a hiding place on the small trees (offshoots) beside the main trees. However, the influence of climatic factors may still be effective even on small trees close to the ground and that would maintain the pressure on insect stages for the subsequent generations.

Results of this study indicated that the arrival and spread of the pest to the intended regions in Al-Anbar Province had no correlation with the nearby infested Provinces. Therefore the possible means of transmission of the pest could be attributed to the small offshoots that are brought by farmers from regions which are already infested by dubas bugs and that would initiate the new spread in the region. The second mean could be attributed to date trading activities. Some date merchants buy quantities of bagged dates from farmers in middle Euphrates provinces, such as Karbala and Babil Provinces, and hold them in open storages for exporting abroad. The date bags may act as a carrier of insect stages from infested region to the new places which then find their ways to move to nearby date palm trees to begin a new infestation and if the surrounding conditions are favorable for development and population increase. Therefore, insect density may reach the damaging levels which were already reported in orchards around date storages in the region. Other means for dubas transmission are still unidentified and need more investigation. However, this pest may not persist in date palm orchards in Al-Anbar because of the mentioned stress factors and that can be assisted by enhancement of agricultural practices for managing date palm orchards and encouragement of sanitation programs that would include cutting and burning of infested leaves. Development and establishment of a reliable monitoring system is essentially needed to identify and map the distribution of this pest and other pests infesting date palm trees in country.

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Tables

Table 1. Regions included in field survey and infestation levels by dubas bug *Ommatisus lybicus* (Debergevin) in Al-Anbar Province during fall 2007 and spring 2008.

Region	Infestation parameter		Notes
	Fall 2007	Spring 2008	
Falluga	0	0	No sign of infestation
Ramadi	1	1	Invisible infestation
Al-Forat	0	2	Presence of honey dew spots (spring 2008)
Hit	4	2	Trees were heavily influenced by honey dew accumulation reduced to scattered spots for spring generation (fall 2007)
Hadetha	2	1	Infestation was reduced for spring generation
Rawa	2	1	Infestation was reduced for spring generation
Al-Qaim	2	1	Infestation was reduced for spring generation

Table 2. Infestation levels by dubas bug on main and ground offshoots of date palm at the left side of the river in Hit region/Al-Anbar Province during the subsequent generations from spring 2008 to fall 2009.

Insect generation	Type of tree			
	Mother (tall) trees		Small side trees (ground offshoots)	
	Parameter	No. of insects/tree	Parameter	No. insects/tree
Spring 2008	1	Less than one	2	1-10
Fall 2008	2	1-5	3	5-10
Spring 2009	1	Less than one	2	1-10
Fall 2009	1	Less than one	3	5-15

Table 3. Infestation levels by dubas bug on main tree and ground offshoots of date palm at the right side of the river in Hit region/Al-Anbar Province during the subsequent generations from spring 2008 to fall 2009.

Insect generation	Type of tree			
	Mother (tall) tree		Small side tree (ground offshoots)	
	Parameter	No. of insects/tree	Parameter	No. of insects/tree
Spring 2008	1	Less than one	2	1-10
Fall 2008	2	1-5	3	5-10
Spring 2009	1	Less than one	2	1-10
Fall 2009	1	Less than one	3	5-15

Table 4. Number of days with dust storms during the activity periods of dubas bug in spring and fall seasons of 2008.

Season/insect generation	Month	No. of dust storms and type				Notes
		Dense	Medium	Light	Total	
Spring	April	4	4	2	10	Storm density may vary during the same day with continuous presence of light dust during the day all over the month
	May	1	3	9	13	
	June	5	4	3	12	
Total		10	11	14	35	
Fall	September	3	3	3	9	One thunder storm occurred in mid-September
	October	0	1	2	3	
Total		3	4	5	12	

Figures

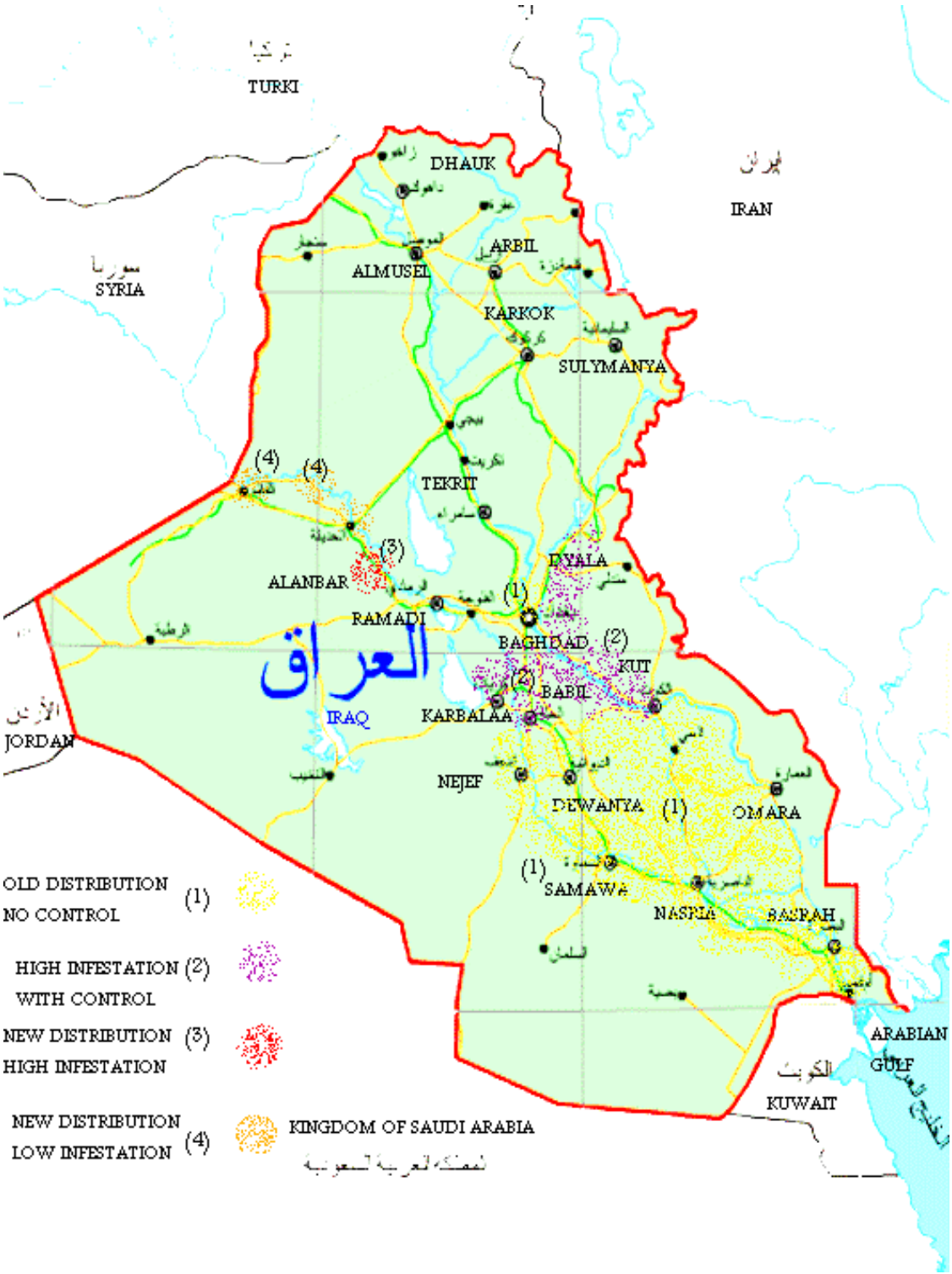

Fig. 1. The old and new spatial distribution of *Ommatisus lybicus* (Debergevin).

Trunk Injection with Neonictoniods Insecticides to Control the Green Pit Scale Insect (*Palmapsis phoenicis* Ramachandra Rao) (*Homoptera*: *Asterolecaniidae*) Infesting Date Palm in Northern Sudan

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Keywords: imidacloprid, thiamethoxam, trunk injection, residues, date palm green pit scale insect, Sudan

Abstract

Small scale field experiments were carried out in Elgaba scheme, and El Golid area during the seasons 2003/2004-2004/2005 to evaluate the efficacy of four systemic insecticides; imidacloprid as Confidor 200SL, Rinfidor 20% SL and Comodor 20% SL and thiamethoxam as Actara 25WG, against the green pit scale insect (*Palmapsis phoenicis* Ramachandra Rao). A trunk injection technique was used. The insecticide thiamethoxam as Actara 25WG was tested at 6, 8 and 10 g/palm while imidacloprid as Confidor 200SL, Rinfidor 20% SL and Comodor 20% SL were tested at 10, 15 and 20 ml/palm. Irrigation was scheduled every 10 days. A Completely Randomized Design with six replicates (one palm=replicate) was used. The insects (all developing stages) were counted (cm^2/leaflet). Eight leaflets from each palm were inspected at biweekly intervals. Dates' yield and quality were determined at harvest. Residue analysis was carried out on dates, soil and intercropped plants twice (at rutab stage and harvesting).

Results indicated that the % mortality (adult and immature stages) were significantly higher in insecticides treatments than the untreated control. Results of residue analysis indicated that no residues of both imidacloprid and thiamethoxam were detected in dates, soil and intercropped plants when treated with the high doses. The higher doses remained effective throughout the experimental period. Date palm treated with the higher doses of tested insecticides developed normally and the dates reached maturity (ripening) and the yield was increased by more than 70% compared with the untreated control. All insecticides checked termites and many other pests, but did not affect mites. This method of application was found highly economical and safe for the users with minimal environmental impacts.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), is considered one of the most important fruit crops, and provides a primary article of food and commerce in the great desert areas of western north Africa to India, and many other subtropical areas. The tree is drought and salt tolerant, and its tasty fruits have high nutritional value and good storage properties. Date fruits constitute the most important agricultural crop in the area and provide highly nutritious food as well as a primary source of income to the majority of the inhabitants. The date palm offers a good food source of high nutritive value (Shinwari, 1993). Furthermore, the date palm tree tolerates relatively harsh climatic and soil conditions under which no other crop may give reasonable returns. In fact, date palm which is an irreplaceable tree in irrigable desert lands, provides protection to under-crops from heat, wind and even cold weather, and plays a big role in combating desertification. Its fruits generate foreign exchange earnings. Its dried fruit bunches, fronds, leaflets fiber and trunks are utilized in many small industries as packing materials in local marketing of fruits and vegetables as well as for many other purposes. The tree and fruit by-products offer an extra income. Timber is produced from stems, while fronds are widely used for thatching, buildings, barring and basketry (household utensils). The date palm tree is cultivated in northern Sudan along the banks of the Nile over a distance of about 900 km.

The total number of trees is about 7-8 million (Osman, 2001; Baballa, 2002; Ahmed, 2005). According to FAO (2005) the mean annual production of dates is 328.2 metric tons. This ranked Sudan as the 7th largest producer of dates among Arab countries.

However, the date palm industry is facing many serious problems, related to low yields, lack of appropriate packing and presentation as well as limited processing of date products. The estimated average yield of bearing date palm tree in the main date growing areas in Sudan is around 20 kg, which is very low compared to the average yield of more than 100 kg in other date growing areas (USA, Qassim in Saudi Arabia and Namibia) (FAO, 2002). The low yields in most countries, including Sudan, are due to soil salinity, poor fertility, insect pests and diseases, lack of maintenance and care due to increasing cost of labour and to shortage of personnel trained in improved cultural practices. As a result of the high cost of production and low prices of the produce, farmers tend to neglect or even abandon their orchards. Although the commonly known insect pests like red weevil and diseases like bayoud have not been reported, in Sudan, the yield of the date palm is affected by many biotic factors among which insects are the most important.

In Sudan, the green date palm pit scale insect, *Palmapsis phoenicis* Ramachandra Rao (*Asterolecanium phoenicis* Rao.) is considered a key pest. This genus, a native of central Asia (Iran), (Ezz, 1973) was not known in Sudan before 1989 when it was first reported by Ali (1989) in El Golid area, as a result of an introduction of some offshoots from Saudi Arabia in 1974. Later, the pest crossed the natural barrier of the Baja desert to invade Elgaba scheme, (150 km south of Dongola, 400 km north of Khartoum) and has become a real threat to date palm cultivation in Northern Sudan. The infested area in El Golid, Elgaba and Old Dongola is about 5000 ha, extending over 60 and 50 km along the west and east banks of the river Nile respectively. The newly reported infestation in Artigasha Island, Burgag scheme and Orbi in Dongola area, Abuhamad in the River Nile State (23000 infested palm trees) and Khartoum State, provides evidence that the pest may continue to spread.

The insect attacks the leaflets, leaf rachis and fruits. It causes chlorosis, degeneration of the leaves, malformation of fruits before maturity leading to losses in production from a range of 30-50 kg to 5 kg per tree (Ali and ElNasr, 1992). The losses may range between 85 and 90% according to infestation rate, cultivar infested and management conditions (Ahmed, 2001, 2004). In the past, and due to lack of indigenous knowledge of the nature of this pest, control efforts were not successful; hence the level of infestation steadily increased.

Based on growing importance of this pest, its serious impact on date production, this study was initiated to investigate the possible control measures for the green date palm pit scale insect. Thus the main objectives of this study were:

1. To determine the efficacy of imidacloprid and thiamithoxam compounds in controlling the date palm green pit insect.
2. To evaluate the efficacy of these compounds against re-infestation by this insect.
3. To study the effects of tested compounds on other insect pests of the date palm.
4. To check the residues of tested compounds in dates, soil, water and intercropped plants.

MATERIALS AND METHODS

Experimental Sites

Small scale experiments were carried out at Elgaba scheme and El Golid area during the seasons (2003/2004-2004/2005) to evaluate the efficacy of four systemic insecticides (belonging to the new group of inecticides, neonictoniods) against the green date palm pit scale insect using trunk injection application techniques. The 'Barakawi' cultivar, the most predominant one was selected. A Completely Randomized Design with 6 replicates (one tree=replicate) was used.

The first experimental site is Elgaba scheme. A farm in the middle of the scheme was selected. The history of infestation dated about 6 years earlier and the estimated loss

in date yield was more than 70 and 80% for 'Barakawi' and 'Gondeilla' cultivars, respectively. Palm age and heights were between 15-20 years, 10-15 m, respectively. Flood method of irrigation from the Nile is conducted monthly via the scheme's main canal. Supplementary irrigation is given using a diesel pump from underground water. Urea was usually added in summer for intercropping fodder crops (maize, durra and legumes). No chemical control has been conducted in the area before.

As a result of the intensive chemical control program in Elgaba scheme in April-June 2004, the experimental site was moved to El Golid area. The location selected was a farm 3 km from the Nile. It was highly infested. The age of trees was between 10 and 15 years with heights ranging between 8 and 10 m. Intercropping with fodder crops was dominant. Urea and cattle manure were applied to fodder crops. Flood method of irrigation from the Nile is conducted during the flood season. Supplementary irrigation is given using a diesel pump from underground water.

The estimated loss in yield was more than 70% for 'Barakawi'. Chemical control was conducted during the extensive control program in this area in 1991.

Cultural Practices Followed before the Experiments

The following cultural practices were usually carried out:

a. Pruning, removal of dead leaves and the lowest row of the highly infested leaves.
b. Raising earth around the palm to facilitate irrigation (every tree is irrigated individually).
c. Pre-watering (24 h) before application (of the insecticides) using diesel pumps from underground water.

Trunk Injection Technique

Holes of 15 cm deep were bored into the trunk and an open end snout metallic tube was inserted. The tube, 25 cm in length and 1.5 cm in diameter was inserted into the hole at an angle of 45° about 1 m above the ground. The tube can hold at least 25 ml of the diluted insecticide (Al-Jbooryi et al., 2001). A developed calibrated "drench mastic" injection gun (used by Fernandez and Gllego, 1997; Filer, 1973) was not available, so a 25 ml measuring cylinder was used for this purpose. When the injection was over, the tube was closed with a tight-fitting flap. Apart from gloves the user also wore a mask for face and eye safety.

The following insecticides were used at the following doses. The injection volume was made up to 25 ml using tap-water.

1. Elgaba Scheme, Season 2003/2004.

1) Thiamethoxam as Actara 25 WG at 10 g/tree (2.5 g a.i).
2) Thiamethoxam as Actara 25 WG at 8 g/tree (2.0 g a.i.).
3) Thiamethoxam as Actara 25 WG at 6 g/tree (1.25 g a.i).
4) Imidacloprid as Confidor 200SL at 20 ml/tree (4 g a.i.).
5) Imidacloprid as Confidor 200SL at 15 ml/tree (3 g a.i.).
6) Imidacloprid Confidor 200SL at 10 ml/tree (2 g a.i.).
7) Untreated control (by injecting with water only).

2. El Golid Area, Season 2004/2005.

1) Thiamethoxam as Actara 25 WG at 2.5 g a.i./tree (10 g product).
2) Thiamethoxam as Actara 25 WG at 2.0 g a.i./tree (8 g product).
3) Thiamethoxam as Actara 25 WG at 1.25 g a.i./tree (6 g product).
4) Imidacloprid Confidor 200SL at 4 g a.i./tree (20 ml product).
5) Imidacloprid Confidor 200SL at 3 g a.i./tree (15 ml product).
6) Imidacloprid Confidor 200SL at 2 g a.i./tree (10 ml product).
7) Imidacloprid Rinfidor 20%SL at 4 g a.i./tree (20 ml product).
8) Imidacloprid Rinfidor 20%SL at 3 g a.i./tree (15 ml product).
9) Imidacloprid Rinfidor 20%SL at. 2 g a.i./tree (10 ml product).
10) Imidacloprid Comodor 20%SL at 4 g a.i./tree (205 ml product).
11) Imidacloprid Comodor 20%SL at 3 g a.i./tree (15 ml product)

12) Imidacloprid Comodor 20%SL at 2 g a.i./tree (10 ml product)
13) Untreated control (by injecting with water only).

A residue analysis was carried out on dates, soil and intercropped plants twice at harvest.

Insects Count

Samples of eight leaflets (two leaflets from each of the four main directions) were inspected at biweekly intervals and examined under binocular microscope. The number of living and dead adult females and immature stages were recorded per three cm^2 of each leaflet (tip, top and bottom). An average per cm^2 was obtained and the following parameters were calculated:

a. Percentage mortality of adult females.
b. Percentage mortality of immature stages.

Pre-spray count was undertaken before insecticide application.

Yield and Yield Components

At harvest, triplicate samples of 50 date fruits were taken at random from each replicate, collected samples were used to assess the percentage fruit maturity (ripening). Sub-samples of ten date fruits were taken to the lab to determine the following parameters:

a. Mean fruit weight (g).
b. Mean fruit length(L) (cm).
c. Mean fruit diameter (D) (cm).
d. The L/D ratio.
e. Percent seed/fruit weight.

Yield in kilogram per palm was determined at harvest. Samples of date fruits, soil and grasses were taken to ARC laboratory at Wad Medani for residue analysis.

Residue Analysis of the Tested Compounds

1. Residues of Thiamethoxam as Actara 25% WG in Date Palms.

Treatment and Sampling. The insecticide was applied by trunk injection at dosage rate 2.5 g a.i. (10 g product) for soil application. The treatments were done on 15/6/2003.

Treated and control samples of date palm fruits, soil, and intercropped plants were taken in three replicates. Samples were collected early season, 25/8/2003 (unripe fruit stage) and at harvest 1/10/2003. Samples collected were brought to the laboratory; the samples were reduced by quartering and kept at -20°C for residue analysis.

Extraction and Clean-Up. The method of residue analysis employed was provided by Syngenta Analytical Department (REM 179.01).

Sub-samples (10 g) each were taken in triplicate from each of the treated and replicates. They include grass and date palm fruits. The material was extracted by homogenizing with 50 ml of a mixture of water/methanol (1:4 v/v) using a high-speed blender for 3 min. The homogenized material was filtered through a Buchner funnel into an Erlenmeyer flask using Celite filter aid. The filtrate was transferred into a 100 ml volumetric flask and brought to volume with the same solvent mixture. An aliquot of 5 ml of the filtered extract was diluted with the same volume of water and cleaned-up by solid phase extraction using disposable solid-phase columns (Bond Elut C-18). Elution was done using water/tetrahydrofuran (1:4 v/v). The elutes were then concentrated to dryness and the residues were taken into the minimum amount of acetone (0.5 ml) and kept for analysis. The extraction of soil samples was done by shaking 50 g soil sample with 200 ml water/methanol mixture (1:4 v/v) for 2 h using a horizontal shaker.

Analysis. Analysis was carried out by thin-layer chromatography (TLC) using ready-made silica gel GF 254 coated plates with a thickness of 0.25 mm (Merck). After spotting of thiamethoxam standard and samples, the plates were developed in a system of isopropanol/toluene (1:1), and visualized under a short wave ultra violet lamp (254 nm). RF values were determined.

2. Residues of Imidacloprid Used as Confidor 200 SL, Renfidor 20% SL and Comodor 20% SL.

Treatment and Sampling. The insecticides were applied by trunk injection; the treatments were done on 10/6/2004 at dosage rates as mentioned in (2.2). Samples contain date palm fruits, intercropped plants and soil were collected from sites treated with high doses; 20 ml product/tree (4 g a.i.). Samples were collected early season, 20/8/2004 (unripe fruit stage) and at harvest 1/10/2004.

Extraction and Clean-Up. For analysis, sub-samples of 50 g date fruits were taken randomly from treated and untreated trees and intercropped plants, and then mixed with 300 ml of a methanol/water mixture (3:1) and allowed to soak for 30 min. Then the sample was homogenized and filtered using 10 g celite as filter aid. The filtered sample was transferred into a graduated cylinder, filled up with methanol to total of 250 ml and homogenized by agitation. An aliquot (100 ml) was removed, transferred into 1000 ml round-bottomed flask and concentrated to about 20 ml using a rotary evaporator.

Clean-up was carried out using 10 g Amberlite XAD 4 resin packed into a chromatography column having an inner diameter of 10 mm. The column was rewetted with methanol and water. All aqueous elutes were discarded. The residues were eluted with 100 ml methanol. This elute was collected and concentrated to dryness and the residues were taken into 0.5 ml acetone and kept for analysis.

Analysis. Analysis was carried out by thin-layer chromatography (TLC); on ready made silica gel GF_{254} coated plates. After spotting of the samples and standard of imidacloprid, the plates were developed in a system of isopropanol/toluene (1:1) and visualized under short wave ultra violet lamp (254 nm).

RESULTS

Trunk Injection Method

1. Insects Count. The mean biweekly total death (adult females and immature stages/cm^2 of leaflet) for the first season 2003/2004 (Table 1) was significantly increased for all insecticides used compared with untreated control throughout the experimental period. The higher doses of insecticides resulted in a higher number of dead insects. Similar results were obtained in the second season (2004/2005) in El Golid (Table 3) when Actara and Confidor as well as Rinfidor 20% SL and Comodor 20% SL were used. The higher doses were superior to the lower doses and the untreated control in the number of total dead insects even 12 weeks after application (the last count).

Results of percentage mortality of adult females and immature stages in the first season in 2003/2004 season (Table 2 and Fig. 1) showed the high efficacy of insecticides as reflected by the 100% mortality of adult female and immature stages during the second week after injection throughout the rest of counts. Similar results were obtained in the second season (2004/2005) as show in Tables 4 and 5 and Figure 2. The new imidacloprid commercial compounds, Rinfidor and Comodor, showed an effective performance similar to Confidor.

2. Yield and Yield Components. Results in Table 6 indicate that all doses of different insecticides significantly affected yield and physical characters of date fruits compared to the untreated control. The higher doses of Actara (10 g) and Confidor (20 ml) resulted in higher fruit weight, fruit length and a lower seed/fruit weight percentage indicating a higher yield. An increase in yield (75%) was observed relative to the untreated control.

Results of the second season 2004/2005 at El Golid (Table 7) confirmed the above mentioned results. All treatments were superior to the untreated control in yield and yield components except the fruit diameter and percentage seed/fruit weight. The higher yields (kg/tree) were observed in the higher doses of different insecticides.

Results of Residue Analysis

Rinfidor 20% SL, Comodor 20% SL and Confidor 200 SL are formulations of

imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazoldin-2-ylideneamine)]. Results of residue analysis indicated that the residues of imidacloprid and its metabolites were below the detection limit (0.09 µg) in all samples analyzed. The Rf of imidacloprid was 0.53. This result indicated that the usage of Rinfidor 20% SL, Comodor 20% SL and Confidor 200 SL at the rate of 35 ml/tree on the date palms are safe for human consumption.

According to the TLC results the Rf value for thiamethoxam standard was 0.56. The minimum detectable amount of thiamethoxam standard was 0.1 µg. The recovery of the method was 85%. No residues of thiamethoxam and metabolites were detected in all samples examined.

DISCUSSION

Trunk Injection Method

In the past, and due to lack of indigenous knowledge of appropriate control measures adopted to control the date palm green pit scale insect, in Sudan, the treatment control efforts were not successful; hence the level of infestation steadily increased. Following intensive research efforts since the year 2000, this study identified systemic insecticides of new generation, neonicotinoid insecticides (thiamethoxam and imidacloprid) such as Confidor 200 SL and Actara 25 WG which proved high effectiveness in controlling the green pit scale insect in the infested trees, through integrated pest management starting with cultural practices or sanitary measures, supplemented with chemical control and impact of natural enemies together with plant quarantine legislations.

Results of insect mortality, yield and yield components obtained from the two seasons (2003/2004 and 2004/2005) indicated that trunk injection was an effective and reliable method for controlling the green pit scale insect. The higher doses treatments; Actara (10 g), Rinfidor (20 ml), Comodor (20 ml) and Confidor (20 ml) were superior to the lower doses and the untreated control in number of total dead insects and percentage mortality, even 12 weeks after application (the last count). Results of percentage mortality of adult females and immature stages showed the high efficacy of insecticides as reflected by the 100% mortality of adult female and immature stages during the second week after injection.

The same findings were obtained by Joseph et al. (2003, 2007) when they tested a trunk Micro-infusion of IMA-jet (imidacloprid) to control the hemlock woolly adelgid (a tiny, piercing and sucking insect) that feed on hemlock twigs. Results indicate that adelgid mortality may occur with 14-28 days and continue for up to 2 years. Hemlocks respond to treatment with a resumption of growth. On the other hand, Smitly et al. (2006) obtained success in controlling emerald ash borer (*Agrilus planipennis* Fairmaire) infesting green ash trees (*Fraxinus pennsylvanica* Marsh) with trunk injection using either imidacloprid (Mauget imidide and Arborjet IMA-jet), or orthen (Acecaps). Results indicate that the Arborjet trunk injection treatments with imidacloprid provided a high level of control (92-100%). Acecaps trunk injection containing acephate, gave 76% control.

Fernandes Cordova and Gallego (1997) found that oaks infested by oak scale insect were cured by injection with prepared capsules of 225 ml Acephate or imidacloprid solution, these insecticides were effective in controlling the scale pest, moreover, they pointed out that more than 79% control of oak scale was obtained when acephate and imidocloprid were injected at rates of 7.5 g a.i. and 0.8 ml per tree respectively. Mathen and Kurian (1977) pointed out that Sevin at a concentration of 1% injected in coconut trunk cased 93% reduction in the infestation level of red palm weevil.

The distribution rates of the thiamethoxam as Actara 25 WG 24 h post injection of 1 and 2 g a.i./palm (Al-Sammariae et al., 2006) showed that it distributed into the sap and it was detected in the injection side and also in the opposite side at different heights. The result indicated that thiamethoxam translocates rapidly into date palm trunk and reaches

the leaves in a short time so it can be drawn from the findings that it can be employed as a fast chemical remedy against most palm insect pests.

As mentioned before, trunk injection requires the use of a systemic insecticide. It is a safe method which affects the pest only without any side effect on natural enemies. Thus the method causes little adverse effects on the environment. When wide spray application with contact insecticides using aircrafts and heavy machinery had been conducted in areas like El Golid the pest recovered within one year and spread from the target area to infest Elgaba scheme and Old Dongola (Obied, 1987; El Fahal et al., 1993; Ahmed, 2003). Furthermore, trunk injection protects the insecticide from adverse climatic factors. If we take into consideration that more than 60% of date palm trees in Sudan are not irrigated, the use of trunk injection is very useful as an alternative solution to soil application method.

This method not only increases user's safety, it also allows the work to be carried out in an extremely economical manner. The dose used is decreased to less than 50% compared to soil application. On the other hand, a three-man team can do the work, one man boring the hole, the second inserting the tube into the holes and the third injecting insecticides and closing tubes. Date palms treated by trunk injection continued to develop normally during the past four seasons. No phytotoxicity has been observed till now in the treated trees. No insecticide residues have been detected either in dates, soil or grasses.

Insecticides are applied through direct injection into the trunk of the date palm to control the red palm weevil (Oihabi, 2003). The influence of injector size, tree species, and season on uptake of injected solution, uptake volume varied among species and injector size, but it usually increased with time. Uptake volume usually decreased as injector diameter decreased. In non-resinous species, the 6 mm (0.24 in) injector gave the best results, but the 4 mm (0.16 in) and the 3 mm (0.12 in) injectors also gave acceptable results. Rubidium content increased over time in sampled needles. One day after injection, Rb^{+} was recovered in all three sections, indicating a homogeneous distribution throughout the tree (Al-Jboory et al., 2001).

Distribution of injected materials throughout the tree is an important factor that may limit the use of the technique because chemicals may accumulate in one part of the tree and not in others. Many factors, including hole depth, injection placement, tree structure, and the number of injections per tree, affect the distribution of solutions (Sachs et al., 1977; Navarro et al., 1992). The injection method also affects distribution. Today, the tendency is to use low-pressure systems comprising individual devices on each injection point in order to control the quantity of material applied on each point (Whiley et al., 1991; McClure, 1992; Navarro et al., 1992). The injector could be reduced in size to 4 mm (0.16 in) in diameter for use in non-resinous species, but length must be increased to 70 mm (2.8 in) to inject trees with thick bark and to reach the xylem without damaging the trunk.

Coniferous species are less effectively injected than angiosperms (Sachs et al., 1977; Reil, 1979). The difference is explained by the wood structure. Conifer xylem is composed primarily of tracheids with greater resistance to water movement than angiosperms, in which the xylem contains large-diameter, vertical vessels. In addition, some coniferous species produce resin in response to tree wounds, which may affect water uptake. For these reasons, trunk injections in conifers are less frequent than in angiosperms, and less information is available on the factors affecting uptake and distribution of injected solutions in these species.

Joseph et al. (2007) stated that uptake occurs when trees are transpiring. The environmental conditions that favor uptake are moderate temperature, adequate soil moisture and high humidity. Generally hot weather and dry soil conditions will results in a reduced rate of uptake. Micro-infusion time varies depending on the season, time of day, environmental conditions and tree health. The average uptake time for hemlock treatment is 30 min. So the correct time of injection (April-June) and cultural practices (removal of the dead leaves and the highly infested leaves in the lowest rows and normal irrigation) recommended by our study are conformed by these findings.

Riad et al. (2007) in Iraq designed a local trunk injector for date palm trees. Many injectors worldwide were manufactured for that purpose, such as Arbocap (www.arbocap.net). The correct use is as follows: 1) preparing the insecticide; 2) charging the containers spring-mechanism, aspirate up to the 40 ml mark and aspirating will be continue a further 10 ml of air; 3) a hole in the trunk (to a depth of 3 cm approx.) will be drilled using a 3.5 mm drill hit for steal; 4) Arbocap will be inserted to a depth of 0.5 cm using a rubber or plastic tipped hammer; 5) pressure will be produced by freeing the Arbocaps spring-mechanism; 6) after complete absorption of the chemical Arbocap will be extracted and the hole will be disinfected.

Imidacloprid and thiamethoxam application through trunk injection is highly economical, as indicated that the best dose of each compound (20 ml/tree and 10 g/tree for imidacloprid as Confidor and thiamethoxam as Actara respectively) was lower than the dose used in soil application method (35 ml/tree and 18 g/tree) by 57 and 55% for imidacloprid and thiamethoxam, respectively.

Therefore the current work supports the belief that there is a remarkable opportunity to inject Actara and other systemic pesticides directly in the palm trunk and it will translocate in appropriate time and can fit well with a date palm pests control program. One hole and one injection at adequate concentration could be enough to distribute Actara in a palm tree within less than 24 h could obtain an efficient curing concentration.

Residues Analysis of the Tested Compounds

The pesticide trunk injection technique which looks environmentally sound is widely demonstrated in date palm pest control programs. The success of this technique is based on the ability of the injected pesticide to translocate in palm sap (xylem and phloem) and provides an adequate concentration levels within appropriate time to keep the pest infestation intensity below the economic threshold.

Rinfidor 20% SL, Comodor 20% SL and Confidor 200 SL are formulations of imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazoldin-2-ylideneamine)]. Results of residue analysis indicated that the residues of imidacloprid and its metabolites were below the detection limit (0.09 µg) in all samples analyzed. The Rf of imidacloprid was 0.53. This result indicated that the usage of Rinfidor 20% SL, Comodor 20% SL and Confidor 200 SL at the rate of 35 ml/tree on the date palms are safe for human consumption.

According to the TLC results the Rf value for thiamethoxam standard was 0.56. The minimum detectable amount of thiamethoxam standard was 0.1 µg. The recovery of the method was 85%. No residues of thiamethoxam and metabolites were detected in all samples examined. Therefore, it is concluded that the use of Actara 25 WG on date palms may be safe if used at the recommended dose. It is known that rapid metabolism of thiamethoxam occurs in plants with two main major metabolites, which are of no toxic effect. In the field soil degradation of thiamethoxam is fast with half-life about four weeks. It gives one major metabolite and finally mineralised to carbon dioxide (Albert and Naeun, 2000).

Results of the date samples in Dammam area in Saudi Arabia collected after 6 weeks from treatment with Confidor 5 G at 100 g/tree and 20 ml/tree to control the red palm weevil, did not show any Confidor residues (Alawi, 1993). The study recommends that these chemicals can be used before six weeks from the date of harvesting of fruits (Sherif, 1994).

Speed translocation of the systemic insecticide thiamethoxam (Actara 25 GW) was investigated (Al-Sammariae et al., 2006) post injection of 4, 8, and 12 g which represent 1, 2 and 3 g a.i. (active ingredient) per plant. Moreover, the concentrations in sap, leaves and dates were monitored at different intervals. The insecticide qualitative and quantitative analyses were determined by employing ELISA and HPLC techniques. The results revealed following the injection of 12 g/palm that Actara moved up in palm trunk sap at a rate of 2.8 m/h and the concentrations in pith and sap which had been collected

from sampling pores (1.4 m above injection point) were 0.64 and 2.939 ppm after 30 and 90 min respectively. This indicates that Actara rapidly moved out of the injection point and diffused into the palm sap. On the other hand the insecticide was detected in leaves (0.093 ppm) and in dates (0.016 ppm) at 240 min post injection and being 0.022 and 0.008 ppm after 33 days respectively.

CONCLUSION AND RECOMMENDATIONS

Conclusion

1. Date palm production and plantations in Sudan considerably deteriorated in the last years as a result of biotic and abiotic stresses, among which the green pit scale is the most important.
2. Trunk injection of imidacloprid (Confidor 200 SL, Rinfidor 20% SL and Comodor 20% SL) and thiamethoxam (Actara 25WG) were highly effective in controlling the green pit scale insect.
3. It proved to be very effective as a protective measure against new infestation.
4. This method of application does not require any expensive machinery or labour for application. It can be safely applied.
5. Trunk injection is a truly effective and reliable method for controlling the green pit scale insect, with minimal environmental impact.
6. This method of application is highly economical and safe for the user and appears to be safe for the beneficial insects.
7. Date palms treated with different insecticides using this method developed normally during four seasons. No phytotoxicity has been noticed in the treated trees.
8. The tested insecticides checked termites and many other pests, but did not affect mites.

Recommendations

Based on the results, the following insecticides with given dosage rates are recommended to control the date palm green pit scale insect, using trunk injection method of application:

1. Actara 25 WG (thiamethoxam) 10 g product/tree (2.5 g a.i.).
2. Rinfidor 20% SL (imidacloprid) 20 ml product/tree (4 g a.i.).
3. Comodor 20% SL (imidacloprid) 20 ml product/tree (4 g a.i.).
4. Confidor 200 SL (imidacloprid) 20 ml product/tree (4 g a.i.).

Removal of the dead leaves and the highly infested leaves in the lowest rows and normal irrigation must be applied.

The common names, trade names, toxicity data and supplier of the tested insecticides are shown in Table 8.

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Tables

Table 1. Mean biweekly total mortality of green pit scale insect from trees treated with different insecticides (using trunk injection method) at Elgaba scheme season 2003/2004.

Insecticide	Dosage rate per palm	Mean no. of dead scales at weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10 g.p (2.5 g a.i)	0.40 (0.9)	6.2 (2.6)^{AB}	9.30 (2.5)^{AB}	9.10 (2.7)^{A}	4.60 (2.6)^{A}	4.70 (2.3)^{AB}
Actara 25WG	8 g.p (2 g a.i.)	0.94 (1.2)	5.6 (2.5)^{AB}	4.00 (2.1)^{ABC}	5.40 (2.5)^{AB}	3.90 (2.1)^{AB}	3.80 (2.1)^{BC}
Actara 25WG	6 g.p (1.5 g a.i)	0.97 (1.1)	4.1 (2.1)^{BC}	2.90 (1.8)^{C}	4.40 (2.2)^{AB}	3.10 (1.9)^{AB}	1.90 (1.5)^{CD}
Confidor 200SL	20 ml (4 g a.i)	1.60 (1.5)	8.8 (3.1)^{A}	6.60 (2.7)^{A}	5.90 (2.5)^{AB}	4.00 (2.1)^{AB}	6.70 (2.7)^{A}
Confidor 200SL	15 ml (3 g a.i)	0.60 (1.0)	2.9 (1.9)^{BCD}	4.10 (2.1)^{ABC}	4.70 (2.3)^{AB}	1.20 (1.3)^{AB}	3.40 (2.1)^{BC}
Confidor 200SL	10 ml (2 g a.i)	1.00 (1.2)	1.4 (1.0)^{D}	3.20 (1.9)^{BC}	3.20 (1.9)^{B}	3.20 (1.9)^{BC}	1.70 (1.5)^{CD}
Untreated control	water only	0.90 (1.2)	1.4 (1.3)^{D}	0.95 (1.2)^{D}	0.73 (1.1)^{C}	0.50 (1.0)^{C}	1.30 (1.3)^{D}
SE±		0.16	0.22	0.17	0.19	0.24	0.17
CV%		23.3	18.0	14.4	15.04	22.0	15.5

Data in brackets were $\sqrt{x+0.5}$.

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 2. Mean biweekly percentage mortality of adult females and immature stages of green pit scale insect from trees treated with different insecticides (using trunk injection method) at Elgaba scheme season 2003/2004.

Insecticide	Dosage rate/palm	% Mortality of scales at weeks after injection					
		0	2	4	6	8	12
A. Adult females							
Actara 25WG	10 g p (2.5 g a.i.)	9.6 (18.1)	100.0 (95.6)^{AB}	100.0 (99.2)^{A}	100.0 (97.2)^{A}	100.0 (97.9)^{A}	100.0 (92.4)^{A}
Actara 25WG	8 g p (2 g a.i.)	13.3 (21.4)	100.0 (91.2)^{AB}	99.3 (85.2)^{BC}	100.0 (91.3)^{AB}	99.3 (85.3)^{B}	98.5 (82.8)^{BC}
Actara 25WG	6 g p (1.5 g. a.i.)	14.3 (22.2)	98.3 (82.5)^{BC}	93.6 (75.3)^{CD}	94.6 (76.5)^{C}	86.8 (68.7)^{C}	92.7 (74.3)^{CD}
Confidor 200SL	20 ml (4 g a.i.)	11.4(19.7)	100.0 (99.6)^{A}	100.0 (99.6)^{A}	100.0 (99.8)^{A}	100.0 (92.9)^{AB}	100.0 (99.2)^{A}
Confidor 200SL	15 ml (3 g a.i.)	13.0 (21.1)	94.6 (76.4)^{C}	99.9 (89.9)^{AB}	100.0 (90.1)^{AB}	90.7 (72.3)^{C}	100.0 (90.0)^{AB}
Confidor 200SL	10 ml (2 g a.i.)	12.0 (21)	91.9 (73.5)^{C}	90.4 (72)^{D}	95.9 (78.3)^{BC}	79.8 (63.3)^{C}	88.3 (70)^{D}
Untreated control	water only	11.0 (20.2)	8.3 (16.7)^{D}	10.7 (19.1)^{E}	14.0 (22.0)^{D}	23.9 (29.3)^{D}	25.1 (30.1)^{E}
SE±		1.99	3.77	2.89	3.72	3.34	2.62
CV%		16.8	8.5	6.5	8.1	8.0	5.9
B. Immature stages							
Actara 25WG	10 g p (2.5 g a.i.)	14.4 (22)	100.0 (99.6)^{A}	100.0 (99.7)^{A}	100.0 (99.4)^{A}	100.0 (95.6)^{AB}	100.0 (98.1)^{AB}
Actara 25WG	8 g p (2 g a.i.)	16.5 (24.1)	100.0 (96.8)^{A}	100.0 (92.5)^{AB}	100.0 (92.4)^{B}	99.8 (88)^{AB}	99.6(86.6)^{ABC}
Actara 25WG	6 g p (1.5 g a.i.)	15.3 (23)	100.0 (93.8)^{AB}	100.0 (91.3)^{B}	99.9 (88.7)^{B}	91.2 (72.7)^{CD}	99.2(85)^{C}
Confidor 200SL	20 ml (4 g a.i.)	13.4 (21.5)	100.0 (99.2)^{A}	100.0 (99.6)^{A}	100.0 (99.7)^{A}	100.0 (99.7)^{A}	100.0 (99.1)^{A}
Confidor 200SL	15 ml (3 g a.i.)	17 (24.4)	99.2 (85.2)^{BC}	100.0 (92.7)^{AB}	95.4 (77.7)^{C}	98.4 (82.7)^{BC}	99.4 (85.7)^{BC}
Confidor 200SL	10 ml (2 g a.i.)	15.4 (23.1)	97.9 (81.7)^{C}	96.7 (79.6)^{C}	87.2 (69)^{D}	78.4 (62.3)^{D}	94.4 (76.3)^{C}
Untreated control	water only	9.3 (17.8)	19.9 (26.5)^{D}	21.4 (27.6)^{D}	21.6 (27.7)^{E}	22.8 (28.5)^{E}	25.5 (30.3)^{D}
SE±		1.46	2.57	2.12	1.45	3.93	3.56
CV%		11.3	5.4	4.4	3.2	9.0	7.7

Data in brackets were arcsine transformed.

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

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Table 3. Mean biweekly total mortality of green pit scale insect from tree treated with different insecticides (using trunk injection method), at El Golid, season 2004/2005.

Insecticides	Dosage rate/palm	No. of dead scales at weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10 g.p (2.5 g a.i)	0.51 (1.0)	7.7 (2.8)^{A}	8.5 (3.0)^{A}	5.7 (2.5)^{A}	3.7 (2.0)^{AB}	5.3 (2.4)^{A}
Actara 25WG	8 g.p (2 g a.i.)	0.71 (1.1)	3.5 (2.0)^{B}	4.3 (2.2)^{BC}	3.1 (1.9)^{CD}	2.4 (1.7)^{BC}	3.4 (1.9)^{BC}
Actara 25WG	6 g.p (1.5 g a.i.)	0.6 (1.1)	2.7 (1.8)^{BCD}	3.1 (1.9)^{C}	2.4 (1.7)^{DE}	1.6 (1.3)^{CD}	2.3 (1.7)^{CD}
Rinfidor 20% SL	20 ml (5 g a.i.)	0.7 (1.1)	6.3 (2.6)^{A}	5.8 (2.5)^{B}	4.3 (2.2)^{AB}	3.5 (2.0)^{AB}	3.9 (2.1)^{AB}
Rinfidor 20% SL	15 ml (4 g a.i.)	0.9 (1.2)	3.3 (1.9)^{BC}	3.1 (1.9)^{C}	2.4 (1.7)^{DE}	2.5 (1.7)^{BC}	3.1 (1.9)^{BC}
Rinfidor 20% SL	10 ml (3 g a.i.)	0.9 (1.2)	1.8 (1.5)^{D}	1.5 (1.4)^{D}	1.7 (1.3)^{E}	1.2 (1.3)^{CD}	2.3 (1.7)^{CD}
Comodor 20% SL	20 ml (5 g a.i.)	1.1 (1.3)	6.8 (2.7)^{A}	5.4 (2.4)^{B}	4.8 (2.3)^{AB}	4.3 (2.2)^{A}	4.3 (2.2)^{AB}
Comodor 20% SL	15 ml (4 g a.i.)	0.9 (1.1)	3.9 (2.1)^{B}	3.5 (2.1)^{C}	2.9 (1.8)^{D}	2.6 (1.8)^{ABC}	3.1 (1.9)^{BC}
Comodor 20% SL	10 ml (3 g a.i.)	1.1 (1.2)	2.1 (1.6)^{CD}	1.8 (1.5)^{D}	2.4 (1.7) ^{DE}	1.9 (1.5)^{CD}	2.4 (1.7)^{CD}
Confidor 200 SL	20 ml (5 g a.i.)	1.1 (1.3)	7.3 (2.8)^{A}	7.9 (2.9)^{A}	4.2 (2.2)^{AB}	3.9 (2.1)^{AB}	3.1 (1.9)^{BC}
Confidor 200 SL	15 ml (4 g a.i.)	0.9 (1.1)	3.5 (2.0)^{B}	4.3 (2.2)^{BC}	1.9 (1.5)^{E}	1.4 (1.4)^{CD}	2.1 (1.6)^{CD}
Confidor 200 SL	10 ml (3 g a.i.)	1.0 (1.2)	2.1 (1.0)^{D}	3.1 (1.9)^{C}	1.7 (1.5)^{E}	1.0 (1.2)^{D}	1.5 (1.4)^{DE}
Untreated control	water only	0.9 (1.1)	0.5 (1.0)^{E}	0.7 (1.1)^{E}	0.5 (1.0)^{F}	1.0 (1.2)^{D}	0.5 (1.0)^{E}
SE±		0.14	0.10	0.11	0.10	0.13	0.12
CV%		24.6	8.7	9.2	8.4	14.1	11.7

- Data in brackets were √x+0.5.
- Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 4. Mean biweekly percentage of adult females of green pit scale insect from trees treated with different insecticides (using trunk injection method) at El Golid, season 2004/2005.

Insecticides	Dosage rate/palm	% Mortality of scales at weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10 g.p (2.5 g a.i.)	23.1 (28.7)	91 (72.8)^{A}	98.6 (88.4)^{A}	95.8 (76.6)^{ABC}	100.0 (98.5)^{A}	99.6 (86.5)^{A}
Actara 25WG	8 g.p (2 g a.i.)	22.9 (28.5)	89.0 (70.8)^{A}	91.5 (73.4)^{ABCD}	87.2 (69.0)^{ABC}	100.0 (98.6)^{A}	100.0 (95.7)^{A}
Actara 25WG	6 g.p (1.5 g a.i.)	27.0 (31.3)	69.4 (56.4)^{BC}	87.7 (69.5)^{ABCD}	65.3 (53.9)^{CD}	100.0 (97.9)^{AB}	99.8 (87.2)^{A}
Rinfidor 20%SL	20 ml (5 g a.i.)	13.6 (21.7)	89.2 (70.8)^{A}	80.0 (97.0)^{ABC}	100.0 (98.0)^{A}	100.0 (99)^{A}	100.0 (97.7)^{A}
Rinfidor 20%SL	15 ml (4 g a.i.)	12.3 (20.5)	61.4 (51.6)^{C}	77.2 (61.5)^{BCD}	100.0 (99.0)^{A}	98.9 (84.4)^{AB}	100.0 (99.2)^{A}
Rinfidor 20%SL	10 ml (3 g a.i.)	20.7 (27.1)	51.6 (45.9)^{C}	72.4 (58.0)^{CD}	98.7 (83.7)^{ABC}	91.2(80.5)^{AB}	97.0 (80)^{A}
Comodor 20%SL	20 ml (5 g a.i.)	23.2 (28.8)	88.7 (70.4)^{A}	97.6 (81.2)^{ABC}	100.0 (94)^{AB}	100.0 (99.2)^{AB}	100.0 (99.5)^{A}
Comodor 20%SL	15 ml (4 g a.i.)	14.7 (22.6)	66.8 (54.8)^{C}	85.3 (67.3)^{ABCD}	100.0 (91)^{AB}	96.0 (78)^{BC}	100.0 (98)^{A}
Comodor 20%SL	10 ml (3 g a.i.)	13.7 (21.7)	50.9 (45.5)^{C}	67.0(54.0)^{D}	78.0 (62.0)^{BC}	92.0 (74.7)^{AB}	100.0 (91.9)^{A}
Confidor 200SL	20 ml (5 g a.i.)	19.9 (26.3)	86.6 (67.7)^{AB}	100.0 (91.0)^{A}	100.0 (99)^{A}	100.0 (99.4)^{A}	100.0 (99.7)^{A}
Confidor 200SL	15 ml (4 g a.i.)	27.9 (31.9)	70.4 (57.0)^{BC}	99.5 (86.0)^{AB}	98.7 (83)^{ABC}	100.0 (99.5)^{A}	100.0 (98)^{A}
Confidor 200SL	10 ml (3 g a.i.)	11.9 (20)	70.6 (57.2)^{BC}	84.8 (67.1)^{ABCD}	89.1 (71.9)^{ABC}	89.2 (71)^{B}	100.0 (91)^{A}
Untreated control	water only	17.3 (24.6)	15.0 (22.0)^{D}	19.0 (24.9)^{E}	25.7 (30.6)^{D}	19.2 (26)^{C}	13.3 (21.4)^{B}
SE±		3.76	3.47	7.13	9.75	6.32	6.26
CV%		25.4	10.5	17.8	21.6	12.8	12.3

Data in brackets were arcsine transformed.

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 5. Mean biweekly percentage mortality of immature stages of green pit scale insect from trees treated with different insecticides (using trunk injection) at El Golid, season 2004/2005.

Insecticides	Dosage rate/palm	% Mortality of scales at weeks after injection					
		0	2	4	6	8	12
Actara 25 WG	10 g.p (2.5 g a.i.)	8.2 (16.6)	100.0 (99.3)^{A}	100 (98.4)^{A}	100 (99.6)^{A}	100.0 (98.1)^{A}	100 (98)^{A}
Actara 25 WG	8 g.p (2 g a.i.)	20.8 (27.1)	97.8 (81.4)^{ABC}	100 (95.2)^{A}	100 (99.6)^{A}	100.0 (96.3)^{A}	100 (96.2)^{A}
Actara 25 WG	6 g.p (1.5 g a.i.)	2.5 (9.1)	96.3 (78.9)^{BC}	100 (92.1)^{A}	100 (99.1)^{A}	98.6 (83.3)^{B}	99.7 (86.9)^{A}
Rinfidor 20% SL	20 ml (5 g a.i.)	23.2 (28.8)	100.0 (99.0)^{A}	100 (95.4)^{A}	100 (98.2)^{A}	100.0 (99.2)^{A}	100 (97.5)^{A}
Rinfidor 20% SL	15 ml (4 g a.i.)	9.9 (18.3)	99.9 (88.3)^{AB}	100 (97.7)^{A}	100 (98.9)^{A}	100.0 (97.8)^{A}	100 (99.2)^{A}
Rinfidor 20% SL	10 ml (3 g a.i.)	4.5 (12.3)	85.5 (67.6)^{C}	100 (90.7)^{A}	98.2 (82.3)^{B}	100.0 (99.3)^{A}	100 (97.2)^{A}
Comodor 20% SL	20 ml (5 g a.i.)	25.6 (30.4)	100.0 (99.5)^{A}	100 (98.5)^{A}	100 (99.3)^{A}	100.0 (99.2)^{A}	100 (98.5)^{A}
Comodor 20% SL	15 ml (4 g a.i.)	1.5 (1.4)	100.0 (99.4)^{A}	100 (98.4)^{A}	100 (96.2)^{A}	100.0 (98.6)^{A}	100 (99.1)^{A}
Comodor 20% SL	10 ml (3 g a.i.)	0.4 (1.0)	97.9 (81.6)^{ABC}	100 (95.2)^{A}	100 (90.9)^{A}	100.0 (98.7)^{A}	100 (99.5)^{A}
Confidor 200 SL	20 ml (5 g a.i.)	25.2 (30.1)	100.0 (99.3)^{A}	100 (99.3) ^{A}	100 (99.6)^{A}	100.0 (99.4)^{A}	100 (98)^{A}
Confidor 200 SL	15 ml (4 g a.i.)	11.7 (20.0)	100.0 (98.9)^{A}	100 (98.8)^{A}	100 (99.4)^{A}	100.0 (99.4)^{A}	100 (99.4)^{A}
Confidor 200 SL	10 ml (3 g a.i.)	12.4 (20.6)	100.0 (97.3)^{A}	100 (98.1) ^{A}	100 (95.3)^{A}	100.0 (99.2)^{A}	63.5 (52.8)^{B}
Untreated control	water only	17.5 (24.8)	22.3 (28.2)^{D}	24.4 (29.6)^{B}	24 (29.3)^{C}	23.2 (28.8)^{C}	24.3 (29.5)^{C}
SE±		4.8	5.23	3.47	2.73	2.66	7.22
CV%		45.3	10.5	6.6	5.2	2.0	14.1

- Data in brackets were arcsine transformed.
- Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 6. Yield and yield components of date fruits from trees treated with different insectices (using trunk injection) at Elgaba scheme, season 2003/2004.

Insecticide	Dosage rate/palm	% ripe fruit	Fruit weight (g)	Fruit length (cm) L	Fruit diameter (cm) D	L/D ratio	% seed/ Fruit wt.	Yield (kg/palm)
Actara 25 WG	10 g.p (2.5 g a.i.)	100.0^{A}	7.2^{A}	4.9^{A}	1.6	3.0	9.3^{D}	109.00^{A}
Actara 25 WG	8 g.p (2 g a.i.)	93.3^{AB}	6.8^{AB}	4.9^{A}	1.5	2.9	11.3^{BC}	85.50^{ABC}
Actara 25 WG	6 g.p (1.5 g a.i.)	85.0^{BC}	6.2^{B}	4.3^{BC}	1.7	2.8	12.7^{B}	81.77^{ABC}
Confidor 200 SL	20 ml (4 g a.i.)	100.0^{A}	7.6^{A}	4.7^{AB}	1.6	3.0	10.0^{CD}	103.67^{AB}
Confidor 200 SL	15 ml (3 g a.i.)	86.7^{BC}	7.4^{AB}	4.6^{AB}	1.6	2.9	11.7^{BC}	63.67^{BCD}
Confidor 200 SL	10 ml (2 g a.i.)	80.0^{C}	7.0^{AB}	4.5^{AB}	1.5	2.8	12.3^{B}	57.77^{CD}
Untreated control	water only	48.3^{D}	4.9^{C}	3.9^{C}	1.5	2.6	15.7^{A}	28.30^{D}
SE±		2.47	0.37	0.14	0.06	0.17	0.56	11.0
CV%		5.0	9.5	5.6	6.5	9.9	8.1	25.1

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 7. Yield and yield components on date fruits from trees treated with different insecticides (using trunk injection method) at El Golid, season 2004/2005.

Insecticides	Dosage rate/palm	% ripe fruit	Fruit wt. (g)	Fruit length (cm) L	Fruit diameter (cm) D	L/D Ratio	% seed per fruit wt.	Yield (kg/palm)
Actara 25 WG	10 g.p (2.5 g a.i.)	100.0^{A}	7.3^{A}	4.6^{A}	1.6^{A}	3.0^{A}	9.3^{FG}	97.3^{AB}
Actara 25 WG	8 g.p (2 g a.i.)	86.7^{BCD}	7.1^{AB}	4.1^{ABCD}	1.6^{A}	2.6^{ABCD}	12.7^{BCDEF}	87.3^{BC}
Actara 25 WG	6 g.p (1.5 g a.i.)	70.0^{EF}	6.9^{ABC}	3.4^{EF}	1.6^{A}	2.1^{CD}	8.3^{G}	79.0^{CDE}
Rinfidor 20% SL	20 ml (5 g a.i.)	$90.0A^{BC}$	5.7^{ABCD}	4.2^{ABC}	1.4^{AB}	3.0^{A}	11.3^{DEFG}	96.0^{AB}
Rinfidor 20% SL	15 ml (4 g a.i.)	83.3^{CD}	5.4^{BCD}	3.7^{BCDE}	1.6^{A}	2.3^{ABCD}	14.7^{ABCD}	86.0^{BCD}
Rinfidor 20% SL	10 ml (3 g a.i.)	65.1^{F}	5.1^{CD}	3.5^{DEF}	1.4^{AB}	2.6^{ABCD}	16.3^{AB}	72.7^{E}
Comodor 20% SL	20 ml (5 g a.i.)	96.7^{AB}	5.9^{ABCD}	4.1^{ABCD}	1.6^{A}	2.6^{ABCD}	9.7^{EFG}	96.7^{AB}
Comodor 20% SL	15 ml (4 g a.i.)	80.0^{CDE}	5.1^{CD}	3.7^{CDE}	1.4^{AB}	2.5^{ABCD}	13.3^{BCDE}	75.7^{DE}
Comodor 20% SL	10 ml (3 g a.i.)	60.0^{F}	4.7^{D}	3.4^{EF}	1.3^{B}	2.8^{ABC}	14.7^{ABCD}	68.0^{E}
Confidor 200 SL	20 ml (5 g a.i.)	100.0^{A}	5.9^{ABCD}	4.3^{AB}	1.5^{AB}	3.1^{AB}	10.0^{EFG}	99.7^{A}
Confidor 200 SL	15 ml (4 g a.i.)	83.3^{CD}	5.3^{BCD}	3.6^{DE}	1.6^{A}	2.2^{BCD}	11.7^{CDEFG}	86.3^{BCD}
Confidor 200 SL	10 ml (3 g a.i.)	78.6^{DE}	4.2^{D}	3.2^{DEF}	1.4^{AB}	2.2^{BCD}	15.3^{ABD}	68.7^{E}
Untreated control	water only	50.0^{G}	4.3^{D}	3.1^{F}	1.4^{AB}	1.9^{D}	18.3^{A}	24.7^{F}
SE±		3.15	0.51	0.18	0.07	0.21	1.13	3.28
CV%		6.8	15.8	8.4	8.2	14.5	15.4	7.1

Means with letter(s) in common are not significantly different at 5 % level according to Duncan's Multiple Range Test.

Table 8. Trade and common names, toxicity and suppliers of the tested insecticides.

Trade name	Common name	Chemical name	Toxicity				Suppliers	
			Acute oral LD_{50} (mg/kg) (Rat)	Acute dermal LD_{50} (mg/kg) (Rat)	Acute inhalation LC_{50} 4 h (Rat)	Skin irritation (Rabbit)	Principal company	Local agent
Actara 25WG	thiamethoxam	3-(2-chloro-thiazol-5-ym ethyl-[1,3,5] oxodiazinan-4ylidene-N-nitroamine	1563	>2000	3720	Not irritant	Syngenta (Switzerland)	SySyngenta (Sudan)
Rinfidor 20%SL	imidacloprid	1(6-chloro-3-pyridyl meth yl)-N-nitroimidazolidin-2-ylideneamine	450	>5000	5323	Not irritant	Agromen chemical Co LTD (China)	Riham International Co. LTD
Comodor 20%SL	imidacloprid	1(6-chloro-3-pyridyl meth yl)-N-nitroimidazolidin-2-ylideneamine	450	>5000	5323	Not irritant	Madmac (Jordan)	Green Deal
Confidor 200SL	imidacloprid	1(6-chloro-3-pyridyl meth yl)-N-nitroimidazolidin-2-ylideneamine	450	>5000	5323	Not irritant	Bayer (Germany)	MADCO

The Population Density and Biological Studies on *Ommatissus binotatus lybicus* in UAE

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Keywords: dubas bug, population density, generations, nymph, leaflet

Abstract

The insect *Ommatissus binotatus lybicus* is one of the most important pests on date palm trees in the UAE. The life cycle and the population density of two generations were studied for the first time in the field during a year in this country.

The results showed that the population density of the first generation was higher than the population density of the second generation of this insect and this differentiation may be according to the rates of temperatures and relative humidity during the year in the UAE, and according to population density of the eggs of this insect. According to this study we could minimize the amount of chemical pesticides which were used for control and the times of control as well.

INTRODUCTION

Dubas bug *Ommatissus binotatus lybicus* DeBergevin (*Homoptera*: *Tropiduchidae*) is one of the most important pests of date palm in the UAE, other Gulf States and Iraq. This insect was recorded for the first time in Iraq in 1922 (Rao and Dutt, 1922; Dowson, 1936). This insect was recorded as a pest in the UAE, Saudi Arabia, Kuwait, Bahrain, Oman, Iraq, Egypt, Lybia, Algeria, and Sudan (Al-Haidari and Al-Hafidh, 1986).

The adults and nymphae of dubas bug on the honey dew of date palm leaves and other vegetation parts of date palm during spring and autumn seasons cause many effects on the palm trees and the quality of dates (Al Haidari and Al-Hafidh, 1986; Al-Hafidh, 1988, 2003, 2009; Anon., 2002). The adults and nymphae produce honey dew during their feeding which is covered by the dust on these areas of the leaf surfaces, these reasons have an effect on the biological activities of infested date palm (Al-Haidari and Al-Hafidg, 1986; Al-Hafidh, 2009).

The life cycle and population density of dubas bug were studied. Moreover, the effect of environmental conditions on this insect are not study in the UAE, so these aspects were studied in the Emirate of Abu Dhabi in the UAE during the spring and autumn seasons and their relations with the effect of temperature and relative humidity, population density, the place of laying eggs by the female adult, and the population density of eggs on the different rows of leaves and the lower and upper of leaf during the period of 2002 to 2004.

MATERIAL AND METHODS

These studies were done on young date palms age (3-5 years) in Abu Dhabi beginning from autumn 2002 to autumn 2004.

Thirty nymphae were used and put in a specific cage to study the life cycle of egg, nymph, adult stages, and sexual rate of dubas bug during the two seasons of autumn and spring during 2002 and 2003.

Ten adult pairs of this insect were used, each adult male and female were put in a cylinder (length 15 cm × diam. 10 cm) made of thin parts of plastic sheets which cover the five live leaflets to study the average number of eggs/female and the adult stage of each female and male before and after the mating period. After each female had laid eggs

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they were selected randomly and put with their infested leaflet in smaller cages of the same shape and material of the cylinder (diam. 2 cm × 1.25 height) and fixed on each leaflet with the eggs on three replicates of shoot cuts of date palm and three replicates of small cages for each tree to study the hatching period.

All the data were recorded monthly such as the number of eggs in each leaf level, period of egg, nymph, adult stages and the sexual rate of this insect on date palm during the spring and autumn seasons. Duncan statistic method and T value were used for the statistical analysis on the results of these studies.

RESULTS AND DISCUSSION

Figure 1 shows the differentiations between the female and male adults, while Table 1 shows the periods of egg, nymph, and adult (male and female) stages in the different generations (two generations). The results showed that there is a significant differentiation in the period of egg stage and the period of each generation on the level 0.001, but there is significant differentiation on the level of 0.05 between the periods of adult stage for both male and female adults during the two generations. While the study showed that there is no significant differentiation in the periods of nymph stage and the number of eggs/female in the two generations, these data agree with the results of Al-Hafidh (1988).

The differentiations of the results during the first and second generations were affected by the differentiations of environmental conditions during the seasons of spring and autumn, and might be according to the biological activities during the growth of date palm during the eight months of dubas bug two generations in the year.

Figure 2 shows the effects of temperature and relative humidity on the population density of this insect during the two generations, while on the same time that the higher average of temperature was 38°C, the relative humidity level was 35% during the period of 15 June to end of August (summer), in winter the temperature was 17°C and the relative humidity was around 50%, so the hatching of eggs in the first generation (spring season) began in April and then the insect stages developed where the mortality of adults began in the first week of August, when the temperature is less than 35°C and the relative humidity more than 40% during the second generation which began in autumn from August to November where the beginning of egg laying and the mortality of adult began later.

The results of this study agree with Al-Hafidh (1988) in general because there were no big difference between the temperature and relative humidity levels during the periods of this study in UAE.

The author found that in some date palm orchards with heavy planting, there were few dubas bug adults alive under the base of the date palm leaves which meant that these environmental conditions might offer better conditions to keep the dubas bug alive longer than the other adults when they live in different environmental conditions, but these few live adults died during December.

So, the author advises to use the mechanical method to clean the date palm trunk as a mechanical method for dubas bug control directly after the harvest period of mature dates and this does not offer longer time for dubas bug adults to live and lay eggs. Then the farmers could minimize the chemical pesticide amounts and exposure period to these pesticides.

The result obtained that there was a significant result in the number of eggs/adult and the number of dubas bug insects on the upper surface of the date palm leaves than the lower surface in all the different levels of date palm leaves at the level 0.05 (Fig. 3), but there was significance in this more specific result of the total number of eggs in this study; the higher numbers of eggs were in the first and second level of date palm leaves (Fig. 4). These results agree with the results of (Al-Haidari and Al-Hafidh, 1986; Al-Hafidh, 1988, 2003).

In Figure 4 the results show the number of eggs (Fig. 5) in each level of leaves. The higher number was in the first level of leaves 29.8%, in the second level of date palm

leaves the level was 23.6%, which means the total number of eggs in these two levels of date palm was 53.4% of the total number of eggs in each date palm tree. Then, if the farmer moves these two levels of date palm leaves mechanically this means that the farmer can control the population density of dubas bugs at least 50% on date palm trees without using chemical pesticides. These results agree with the results of Al-Haidari and Al-Hafidh (1986) and Al-Hafidh (1988).

So the farmer could minimize the cost of date production, environmental pollution, and any side effect of pesticides on the date palm production. These results could be applied in all the countries which produce dates over the UAE, Gulf states and elsewhere.

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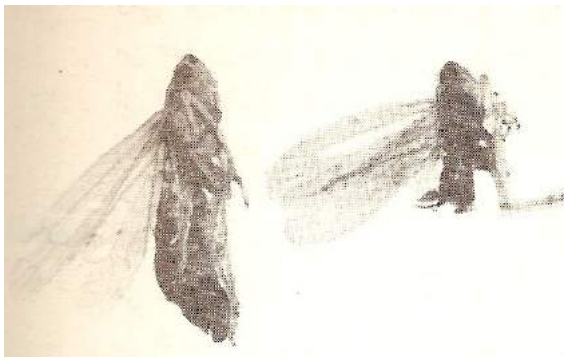
Tables

Table 1. The life cycle data of dubas bug in Abu Dhabi.

Average insect stage	Average of generation periods (days)		Significant values (T value)
	1st generation	2nd generation	
Egg stage	136.5	57.8	***
Nymph stage	41.6	42.9	N.S.
Adult stage			
Female	11.3	16.2	*
Male	5.8	8.2	N.S.
No. of eggs/female	84.57	116.6	**
Total no. of eggs/leaf	58.1 (68.8%)	94.8 (81.3%)	*
Generation period	189.4	116.9	**
No. of sex ration	22.1:13.9	21.8:22.6	*
Female:male	(1.6:1)	(1.04:0.96)	

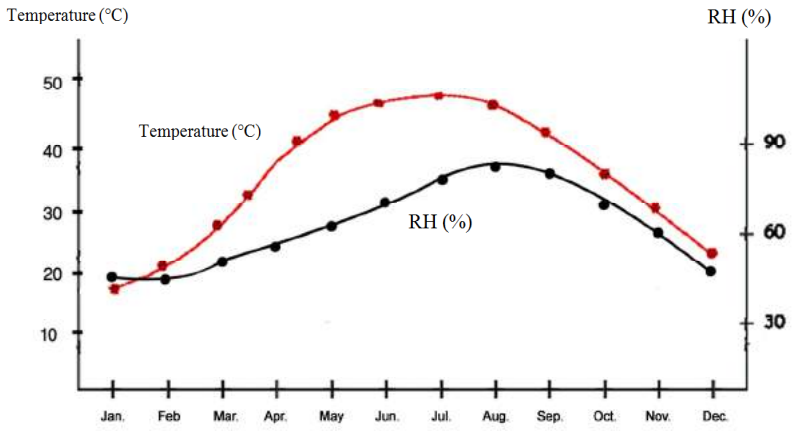
N.S.: not significant; *: significant 0.05; **: significant 0.01; ***: significant 0.001.

Figures



Male Female

Fig. 1. The female and male of an adult dubas bug.



The average of temperature and relative humidity

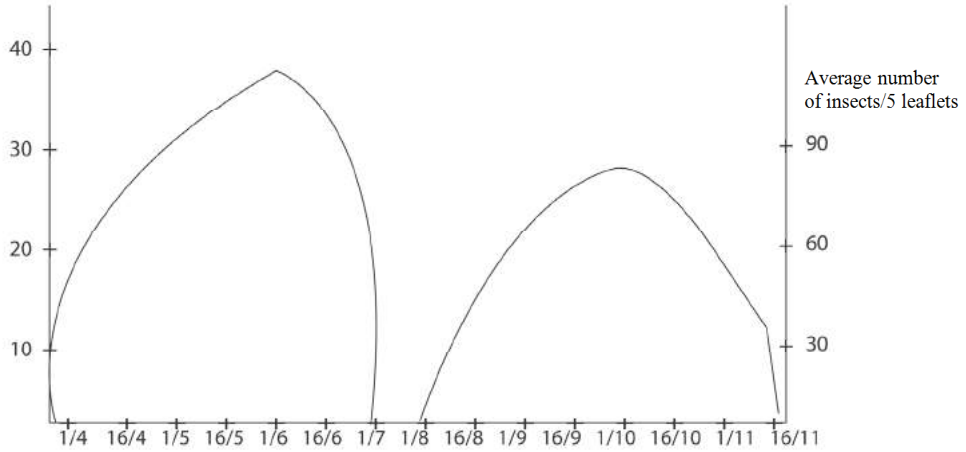
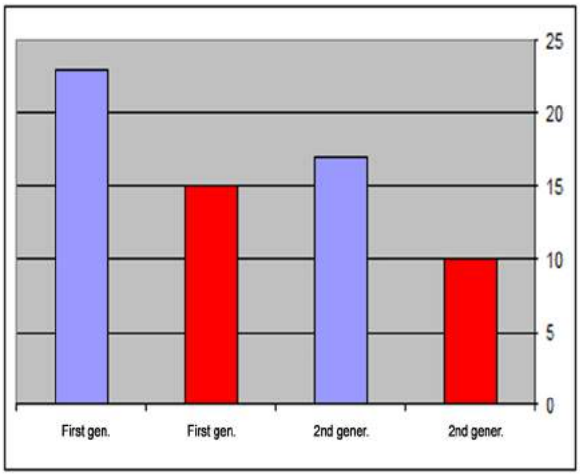


Fig. 2. The average number of insects per five leaflets during the two generations.

Average no. of eggs
Average no.of female

Average no.
of eggs and
female/5 leaflets



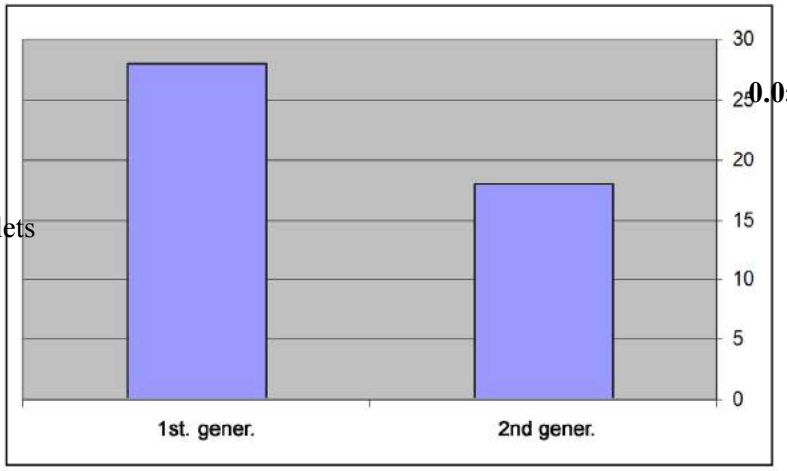
0.01
0.05

N.S.

0.01

N.S.

Average no.
of adults/5 leaflets

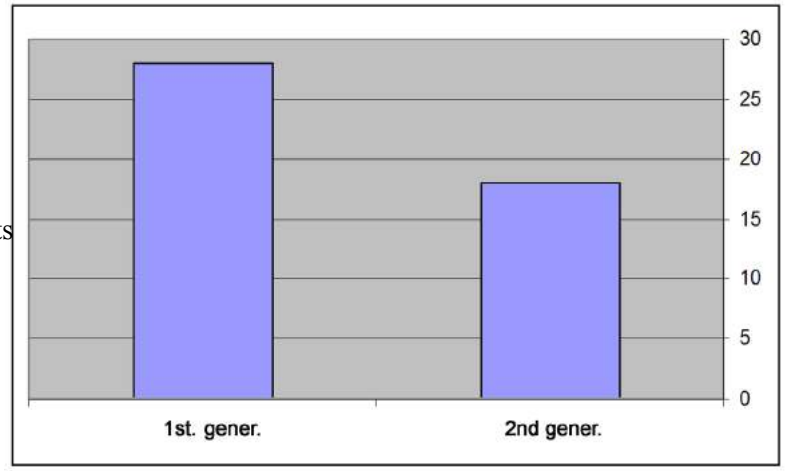


0.05

0.01

0.05

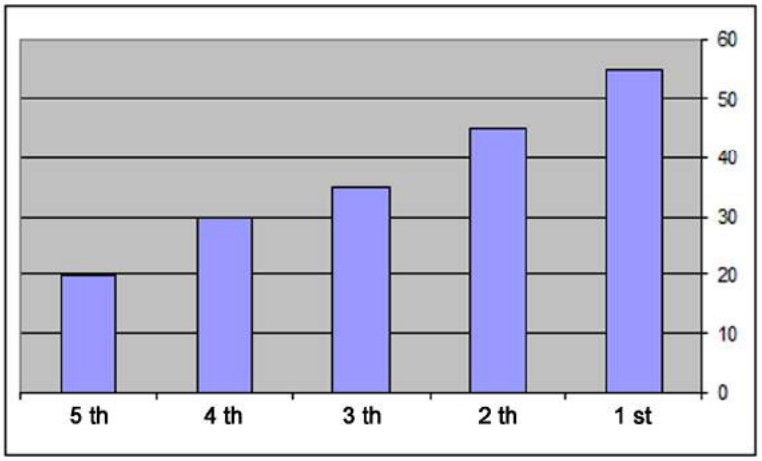
Average no.
eggs/5 leaflets



N.S.

Fig. 3 : showed the average number of adult females and eggs on the upper and lower surfaces during the two generations .

Average no.
of eggs/
5 leaflets in
Different leave
levels



0.01
0.05
N.S.

Upper and lower surfaces of different leave levels
Fig. 4 : Showed the average number of eggs on the different level of leave

Fig.5 : Showed the place of laying eggs by female in the tissues of date palm leaflet .

Test of In Vitro Antagonism of Some Strains of *Paenibacillus polymyxa* towards Strain of *Fusarium oxysporum* f. sp. *albedinis* Agent of the Fusariose of the Date Palm

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Keywords: wheat, *Triticum durum*, Algerian soil, rhizosphere, bayoud, ERIC genotype, biological control

Abstract

The effect of *Paenibacillus polymyxa* (syn. *Bacillus polymyxa*) on control of phoenix, bayoud caused by *Fusarium oxysporum* f. sp. *albedinis* was investigated. In an in vitro assay, seven strains of *P. polymyxa* (SGK2, SGK20, SGD1, SGZ8 and SGH1) were tested against *Fusarium oxysporum* f. sp. *albedinis.* The antifungal effect of our strains was studied on PDA media and King B under illumination and in the dark. Strains *P. polymyxa* SGT12, SGD8 and SGD1 have the ability to inhibit the *F.o.a.* on two media PDA and King B in the dark. In contrast strain SGZ8 inhibits the fungus only in King B medium. Unlike strains SGK2, SGK20 and SGH1 have the ability to inhibit the *F.o.a.* on the PDA medium. The test of confrontation on King B under illumination has not allowed the selection of strains *P. polymyxa* inhibitory *F.o.a.* It seemed interesting to note that inhibition of *F.o.a.* could remain over time on PDA media and King B.

These results showed that *P. polymyxa* is potentially a biocontrol agent for use in controlling of *Fusarium oxysporum* f. sp. *albedinis* in vivo.

INTRODUCTION

The date palm is for our country an essential tree not only for its economic importance but also because it forms an integral part of our religious inheritance, historical and cultural, which explains all the actions taken by our government to develop this culture in order to facilitate the establishment of Saharan populations and intensify relations between the Sahara and other regions of our country. However, the average yield per palm remains low in our country compared to that obtained in the United States and Egypt (Benmahcene, 1998). The cultivation of this tree is threatened by pests and diseases which seriously reduce the crop quantitatively and qualitatively. Among the diseases of Phoenix, bayoud caused by the *Fusarium* fungus, *Fusarium oxysporum* f. sp. *albedinis*, risks destroying almost all cultivars of date palms and especially the cultivar 'Deglet-Nour'.

Biological control represents an attractive alternative for the future because of the many concerns about pesticides use. Ideally, an agent of biological control of fungal root pathogens should exert a sufficient amount of antagonistic activity in the rhizosphere to significantly reduce root disease symptoms.

Paenibacillus polymyxa (=*Bacillus polymyxa*, Ash et al., 1994), a common soil bacterium belongs to the group of plant growth-promoting rhizobacteria (PGPR) (Timmusk et al., 1999; Selim et al., 2005). A range of activities has been found to be associated with *P. polymyxa* treatment, some of which might be involved in plant growth promoting (Timmusk et al., 2003). Indirect promotion of plant growth occurs when PGPR antagonizes or prevents the effects of phytopathogens or deleterious micro-organisms.

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Most mechanisms proposed to explain indirect growth promotion suggest that the active principle may be a secondary bacterial metabolite which antagonizes pathogens.

P. polymyxa is known to produce antibiotic compounds and inoculation with *P. polymyxa* suppressing several plant pathogens (Mavingui et al., 1992; Smid et al., 1993; Beatty and Jensen, 2002; Barkley et al., 2001; Timmusk, 2003; Haggag, 2007). It has been isolated from the rhizospheres of white clover, perennial ryegrass, crested wheat grass (Holl et al., 1988) and green bean (Petersen et al., 1996) cucumber (Roberts et al., 2005) and garlic (Kajimura and Kaneda, 1996). Induced resistance can also be as a result of root colonization by PGPR (Timmusk et al., 2003). The latter response is called induced systemic resistance (ISR), and has been shown to protect against disease in several plant species (Timmusk et al., 1999, 2003).

The objectives of the present work were to proceed with the selection of strains of *P. polymyxa*, *Fusarium oxysporum* f. sp. *albedinis* antagonists and this from a collection of 111 strains, because to our knowledge, the use of this bacterial species in biological control against the *Fusarium oxysporum* f. sp. *albedinis* had never been done.

MATERIALS AND METHODS

Bacterial Strains

1. Reference Strains and Collection. Strains *Rhizobium gallicum* R602^T, *Sinorhizobium meliloti* 1021, *Rhizobium* sp.YAS34, *Pseudomonas brassicacearum* NFM421 and *E. coli* EDCM367 obtained from collections of LEMIR (CNRS, France) were used in this study. They were used to compare their activity to those of strains *P. polymyxa* isolated in Algeria for testing in vitro confrontation towards the *Fusarium oxysporum* f. sp. *albedinis*.

2. Strains Isolated from the Rhizosphere of Durum Wheat in Algerian Soils. Among the collection of 111 strains *P. polymyxa*, we selected with a criterion of diversity, seven strains to the test of antagonism (Table 1). *P. polymyxa* strains were isolated from the rhizosphere of different soils cultivated with durum wheat by an immuno-enzymatic method "immuno-trapping" (Guemouri, 1992). The strains were identified and their diversity was searched by using various methods API, RFLP and gene sequencing of ARNr16S (Athmani-Guemouri, 2006). Each strain belongs to a different ERIC group (Athmani-Guemouri et al., 2000).

The Fungus

The aggressive fungus used in this work came from the Institut National Agronomique (INA) of El-Harrach (Algeria). It is of *Fusarium oxysporum* f. sp. *albedinis*.

Test of In Vitro Antagonism

The antifungal effect of our strains was studied on agar medium in a petri dish (Weller et al., 1985). Each bacterial strain was seeded at a given point on the edge of the petri dish containing PDA (potato dextrose agar) medium or King B and incubated 48 h at 30°C.

A square of 7 mm diameter, cut to the punch in the PDA agar containing the fungus is deposited at the center of the box containing the bacteria. The boxes were incubated in the dark for 7 days at 28°C. For each strain tested, three replicates were performed. We have also cultivated the fungus in the absence of bacteria, it is the control.

Effect of Light on the Inhibitory Activity

A test confrontation of seven strains of *P. polymyxa* with *F.o.* f. sp. *a.* has been achieved in vitro by incubating the boxes under illumination.

RESULTS AND DISCUSSION

Test of In Vitro Antagonism

The results are summarized in Figure 1. From Figure 1, there are three strains *P. polymyxa* that have the ability to inhibit the *F.o.a.* on two media PDA and King B in the dark. These are the following strains: SGT12, SGD8 and SGD1 (Fig. 2).

In this case, the combination of different molecules (siderophores, antibiotics and chitinolytic enzymes) could be involved (Dijksterhuis et al., 1999). This result corroborates with the ones found by Mavingui (1992), although the fungus tested was different.

In contrast, strain SGZ8 inhibits the fungus only in King B medium (Fig. 3), it could be by production of siderophores.

Unlike strains SGK2, SGK20 and SGH1 which are inhibitory on the PDA medium (Fig. 4) other inhibitory substances such as antibiotics would be produced (Thomashow and Weller, 1990). These latter have a fungistatic effect towards certain special forms of *F.o.a.* (Sedra and Bah, 1993).

The inhibitory effect observed only on PDA could also be linked to the presence of chitinases.

Effect of Light on the Inhibitory Activity of Our Strains

We also tried to see the effect of light on the inhibitory activity of our strains. The results listed in Table 2 show that the strains SGK2 and SGD1 have an antagonistic effect on PDA in darkness, then they are not under illumination. Other strains are inhibitors of *F.o.a.* in the absence and presence of light; it is SGK20 strain, SGT12, SGD8 and SGH1. The test of confrontation on King B under illumination has not allowed the selection of strains *P. polymyxa* inhibiting *F.o.a*. It seems that the regulation of certain genes involved in these activities is under dependence of light.

Persistence of the Inhibitory Effect over Time and Reproducibility of Results

In order to ascertain whether the antagonistic effect of our strains persists over time or not, we proceeded to re-incubate the same boxes. After 30 days of incubation, we found that *Fusarium oxysporum* f. sp. *albedinis* which stands alone without the presence of *P. polymyxa* could invade the entire box by cons; this invasion was not observed when the fungus is present with the bacteria. This was a barrier that the fungus could not cross (Fig. 5).

Finally, the high reproducibility of results seemed worth noting. Indeed, similar results were obtained in three independent experiments, which show the reliability test.

Note that the reference strains and collection have no effect on *F.o.a.* tested in this study (Fig. 6).

CONCLUSION AND PERSPECTIVES

The use of micro-organisms to control plant diseases offers an attractive alternative to the use of synthetic chemicals (Roberts et al., 2005). The abundance of a beneficial strain of micro-organism in the vicinity of plant roots may suppress plant pathogens without producing lasting effects on the rest of the soil microbial and plant communities.

Knowing the diversity of populations *P. polymyxa* isolated from the rhizosphere of five soils cultivated with durum wheat, helped us to select seven representative strains SGK2, SGK20, SGT12, SGD8, SGD1, SGZ8 and SGH1 and were tested in antagonism tests against *Fusarium oxysporum* f. sp. *albedinis* serious pathogen of the date palm.

P. polymyxa is gaining recognition for biocontrol in a variety of plants, even though it is mainly used as a seed protectant and antifungal agent (Timmusk et al., 2003).

The results of the test of in vitro confrontation in the dark enabled us to conclude that the inhibition of growth of this fungus by bacteria tested on PDA medium and King B could be due to a combination of several molecules (antibiotics, hydrolytic enzymes

and siderophores). The strains with an inhibitory effect only on the PDA medium would produce either antibiotics or hydrolytic enzymes or both at the same time. The only inhibitory effect observed on King B medium, could be attributed production of bacterial siderophores. Alternatively, direct competition for food is a plausible scenario. We found that inhibition of *F.o.* f. sp. *a*. could remain over time on PDA media and King B. This result can be explained by the stability of substances produced by *P. polymyxa*.

The test of confrontation on King B under illumination was not possible to select strains *P. polymyxa* inhibitors of *F.o.* f. sp. *a*. It seems that the regulation of certain genes involved in these activities is under dependence of the light. It seemed interesting to note the high reproducibility of results. Finally, the results found show that the reference strains and collection tested in this study are unable to inhibit the *F.o.a*.

These results open serious perspectives in the development of integrated control against this fungus. The *P. polymyxa* studied in this work could be good candidates for tests in vivo.

Two soils were sampled in two distant fields near Tiaret (200 km south-west of Algiers). These fields (K>100 and T>100) have been constantly under wheat cultivation since Roman times, and we estimated that wheat had been grown there for about 2000 years (Laumont and Erroux, 1961). Another soil was sampled in a field at the Institute for the development of major crops (D 70) (IDGC), a governmental agronomic experimental station in Algiers. This field had been cultivated since 1920 in rotation including wheat every 2 years. The last two soils were sampled in two agricultural fields located in Hamiz (10 km east of Algiers). These soils had been cultivated in rotation including wheat every 2 years for 5 and 26 years respectively (H5 and Z26).

The inhibitory effect was assessed by the formation of a zone of inhibition. A strain is declared negative if no zone of inhibition was observed.

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Tables

Table 1. Strains of *Paenibacillus polymyxa*, ERIC-PCR group, origin and age of culture in durum wheat (Athmani-Guemouri et al., 2000).

Site	Cultivation of wheat for*	Strain	ERIC genotype
Tiaret	>100 years (K>100)	SGK2	1
		SGK20	4
Tiaret	>100 years (T>100)	SGT12	2
I.D.G.C.	70 years (D 70)	SGD8	9
		SGD1	10
Hamiz	26 years (Z 26)	SGZ8	17
Hamiz	5 years (H 5)	SGH1	19

* Two different soils with a long history of wheat cultivation, and three soils cultivated more recently with wheat were chosen (Guemouri, 1992). The five soils were sampled in three different wheat cultivation areas in Algeria.

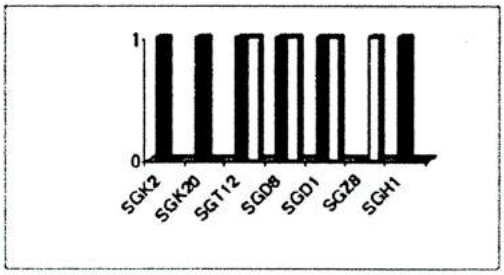
Table 2. Effect of inhibitory strains *P. polymyxa* towards the *F.o.a.* on PDA medium and King B. Incubation under light and darkness at 28°C for 7 days.

Strains	Medium			
	PDA under illumination	PDA in the dark	King B under illumination	King B in the dark
SGK2	-	+	-	-
SGK20	+	+	-	-
SGT12	+	+	-	+
SGD8	+	+	-	+
SGD1	-	+	-	+
SGZ8	-	-	-	+
SGH1	+	+	-	-

Figures

■ : PDA medium

□ : King B



0: any area of inhibition
1:diameter of inhibition zone ≥1 cm

Fig. 1. Effect inhibitor of the 7 strains *P. polymyxa* towards the *F.o.a.* on the PDA media and King B. Incubation in the dark at 28°C for 7 days. Regarding the diameter of inhibition zone, Girardin et al. (2002) consider that when it is greater than or equal to 1 cm, the bacterial strain is inhibiting the fungus, when is between 0.5 and 1 cm, the bacterial strain is weakly inhibitory and when any area of inhibition appears, the strain has no effect on the fungus. In this work the zone diameter of inhibition was always greater than 1 cm including the medium King B.

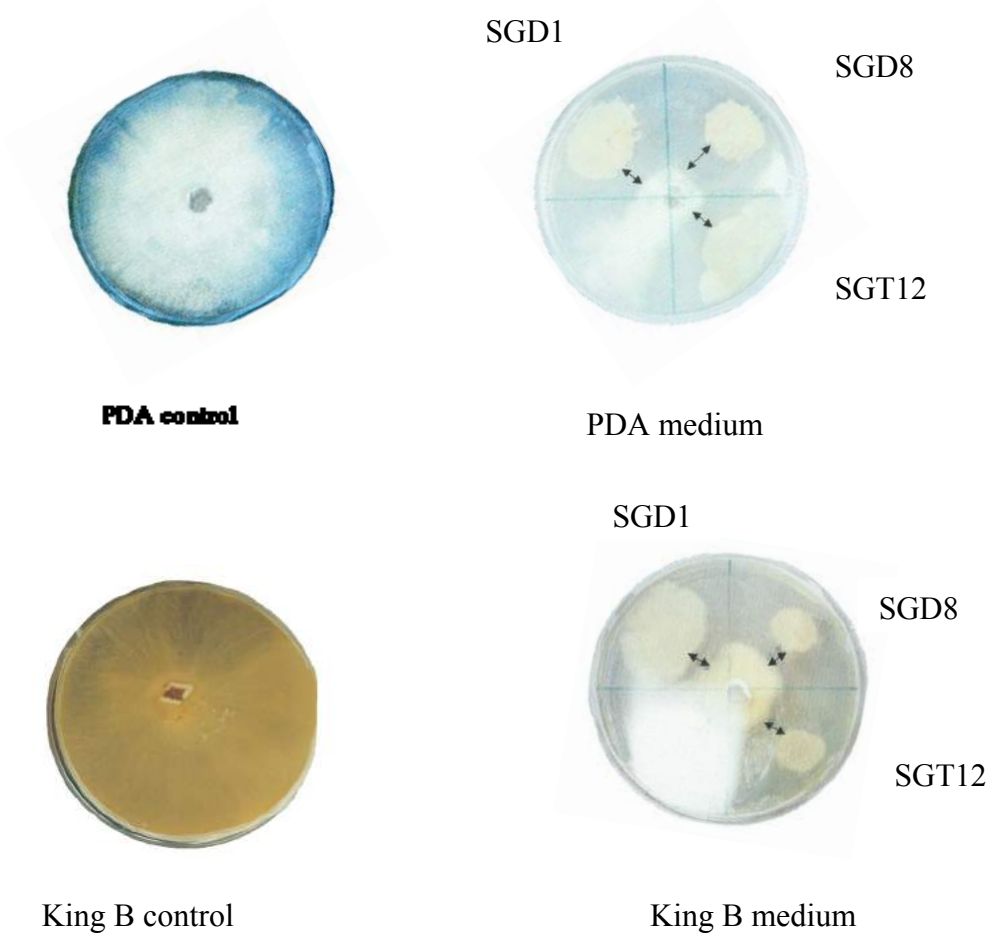
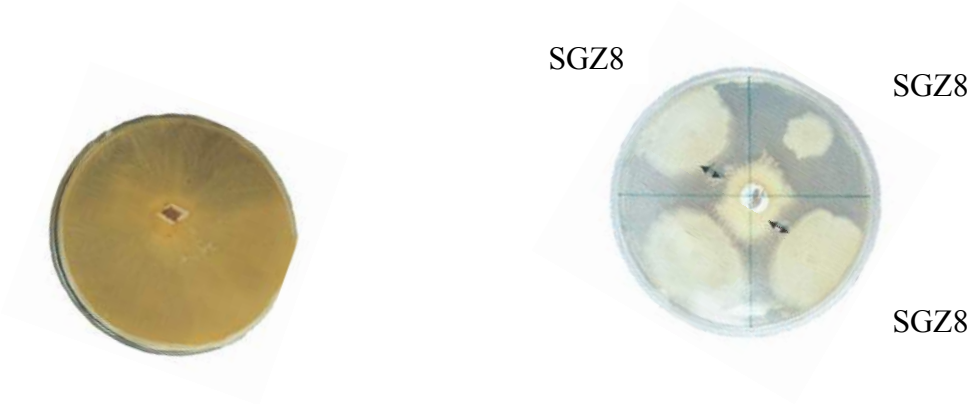
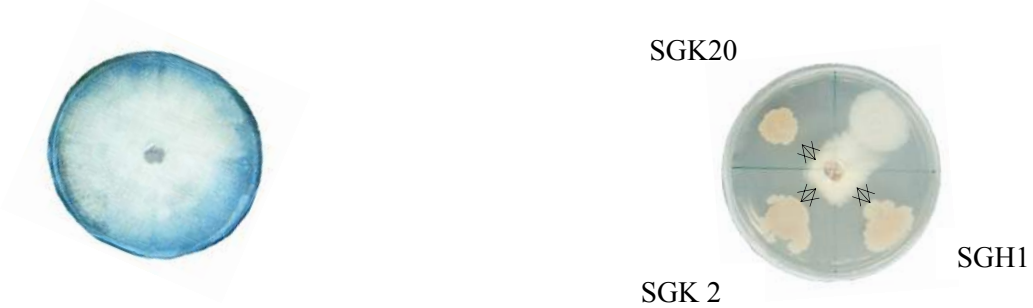


Fig. 2. Inhibition of growth of *F.o.a.* by strains SGT12, SGD8 and SGD1 after 7 days incubation at 28°C on PDA medium and King B in the dark. ↕: corresponds to the zone of inhibition.



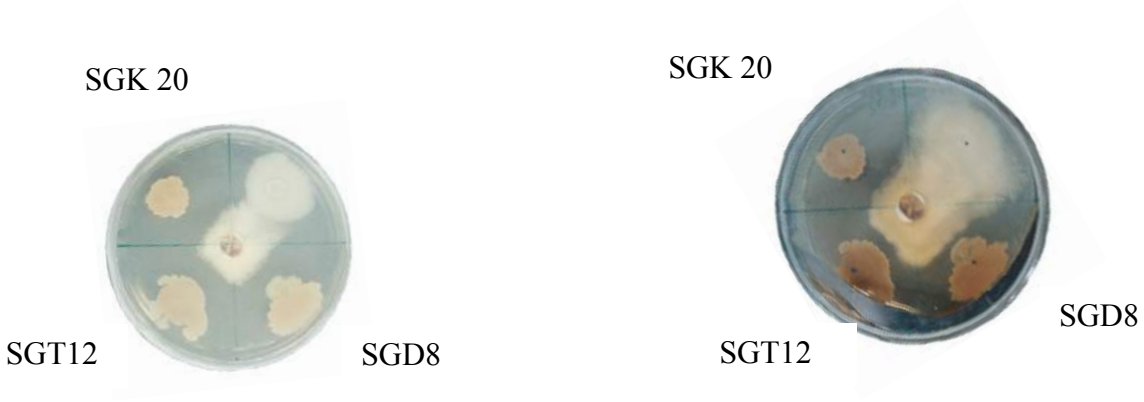
King B control King B medium

Fig. 3. Inhibition of growth *F.o.a.* by the strain SGZ8 after 7 days incubation at 28°C on King B medium in the dark.



PDA control PDA medium

Fig. 4. Inhibition of growth *F.o.a.* by strains SGK2, SGK20 and SGH1 after 7 days incubation at 28°C on PDA medium in the dark.



PDA medium after 7 days PDA medium after 30 days

Fig. 5. Persistence of the inhibitory effect of strains SGK20, SGT12 and SGD8 towards the *F.o.a.* on PDA media after 30 days of incubation at 28°C.

PDA control PDA medium

Fig. 6. The reference strain *Rhizobium gallicum* R602^T has no effect on *F.o.a*. tested after 7 days incubation at 28°C on PDA medium in the dark.

Different Methods to Combat the Red Palm Weevil Compared to Modern Injection Method by Amended Italian Machine (Endo Palm)

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Keywords: date palm, red palm weevil, control, injection, pesticide

Abstract

Gas control pills do not give the desired results, and there is difficulty in use, especially when there is a large hole in the palm which requires to be closed tightly, and sometimes the employee is exposed to gas fumes which is internationally forbidden to be used.

Outer spray has been used since the start of injury in Arab countries, but did not succeed due to the difficulty of pesticides to kill insects, but it is considered to be complementary to that of control programs to kill larvae and pupae and insects between the folds of the full cut Jerid.

Burning the injured palms did not give good results, due to the flight of insects when they feel heat.

Injection by pipes gives moderate results in the case of some medium new infections, and have been found quickly, and placed in only one wound. But in the case of multiple sites of injury or injury under the head, as well as wounding of a tall palm under its head, it will be a very difficult treatment to make holes in palm parts, as well as the difficulty of hammering in the farms which have no electricity for the use of an electrical driller, and took a long time in injection by pipes.

Moreover, the environment is affected by pouring pesticide outside the palm and the lack of access to all pesticide sometimes eccentric within the palm as well as all parts of the palm, compared with injection by the amended injection machine (Endo Palm) ,which is characterized by the following:

1) **Quick usage: ability of injection of 30-50 palms per day.**
2) **Ease of use.**
3) **Preserving the environment and worker and human health.**
4) **Access of the pesticide, by thanking to Allah, to all parts of the palm and the phases of the insect.**
5) **The ability to treat so tall injury palms at its head.**
6) **Saving of pesticides and the efficiency of injection and the efficiency of controlling.**
7) **Giving hope to farmers in the possibility to have an effective way to combat the weevil.**

We already have injected palms 3 years ago, and no injury returned back to it till now.

We have injected chemicals and a natural oil of Dr. Shams. Also we injected isolated bacteria from the palm weevil insects, which is a compound of Dr. Ahlam El-Fazeery. Also a compound of Dr. Swify which is isolated fungus from the palm weevil insects. All these compounds, thanking to Allah, gave very good results. Besides private compounds by Dr. Nabawy, compounds of my own will be recorded as a patent.

INTRODUCTION

I was born among the data palm trees in Edko (Behera Governorate, Egypt), the town of a million palm trees. In Edko, Rashid and neighboring areas, there are more than seven million palm trees. I spent my childhood and youth in my father's palm ranch and

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was raised with well-established criteria about the sacred rank of the palm tree in our souls and you should protect it from any harm. In Egypt, the data palm trees are considered as one of the main sources of the national income. On the other hand, Bedouins and many other Egyptians depend totally on data palm trees as the only backbone of feeding, living, and earning money to raise and wed their sons and daughters. Therefore, from an early time of my life and till now, I am eagerly interested in anything related to the palm tree, its health, diseases, insects and productivity.

I am a member in the national campaign for the prosperity of the date palm trees in Egypt; attended nearly, all the international and local conferences on palm trees, moreover, I have organized two conference in Edko about problems and solutions that affect the productivity of the date palm trees.

Since the occurrence of the red palm weevil, *Rhyncophorus ferrugineus* (Olivier) and its drastic threat to date palms, *Phoenix dactylifera*, in the eighties (in Gulf States) and nineties (in Egypt), an unusual attention and care has been given to palm trees in the middle east region.

The red palm weevil is considered one of the most dangerous insect pests infesting palm trees in over 35 countries in Africa, the Mediterranean basin, East Asia, and part of Europe and is referred to as the palm cancer (Tofailli, 2010) or the palm AIDS.

The paper gives an overview on the red palm weevil conventional control measures and Nabawy endotherapic injection method. The red palm weevil conventional control measures can be summarized as follows:

1. agricultural control and field sanitation procedures.
2. pheromone-keromone traps.
3. application of the external and internal quarantine regulations.
4. chemical control: by applying insecticides as:
    a. periodic sprays to kill larvae, pupae and adults between folds of the full cutjerid.
    b. fumigation by gas pills; this measure does not give the desired result, especially with large holes in the palm which require to be tightly closed and sometimes the employee is exposed to gas fumes which are forbidden internationally to be used.
    c. injection by pipes gives moderate control results in case of medium new infestations with single injury site. But in the case of multiple sites of injury or injury sites under the head of relatively short or so tall palm trees, it will be a very difficult treatment to make holes in palms. Also, this method faces the great difficulty of hammering the palms in farms with no electricity for the use of an electrical driller. Moreover, injection by pipes takes a long time and the injected insecticide does not reach all parts of the palm; not to mention the environmental pollution by pouring the injected insecticide outside the treated hole.

Based on all the above mentioned disadvantages of the insecticide injection by pipes, Nabawy (2010) has patented an injection machine (Endo Palm) which proved its efficiency as a very promising injection machine for palm trees to control the red palm weevil. This machine is characterizes by the following advantages:

a. It takes a very short time to inject the required amount of the insecticide, its ability of injection ranged between 30-50 palms per day.
b. Ease of use.
c. No problems or difficulty to treat tall injured palm trees and their heads.
d. No loss of the injection insecticide during injection.
e. No environmental pollution and provides complete safety for workers during application.
f. The injected insecticide reaches all parts of the palm tree.
g. It gives hope to farmers in the possibility of controlling the red palm weevil effectively.

Field applications of the endotherapic method by means of the tree vital machine (Endo Palm) on 3500, 600 and 400 palm trees in Italy, Saudi Arabia and Egypt, respectively has emphasized the highly efficient role of such a promising injection machine as a curative or preventive tool against the palm cancer, *R. ferrugineus*.

Up to date, all injected palm trees have recovered and appear healthy.

By co-operation with Dr. Nabawy, several compounds of his own and others of mine, recommended insecticides, natural oil (Dr. Shams), entomopathogen bacterium, originally isolated from red palm weevil (Alfazairy et al., 2003), and a fungal compound (Dr. Swify), all of these compounds gave very good promising results.

Based on the present field observations, I would like to suggest certain points. Hopefully, they may contribute to the integrated strategy of controlling the "palm AIDS" the red palm weevil where it is present:

1. An Arabic international center for facing any problem related to the red palm weevil.
2. Such a center may provide financial aid for developing countries to buy the promising tree vital machine "Endo Palm".

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My deep gratitude and thanks to all the following for their valuable and generous help in the course of the present investigation: the Ministry of Agriculture-Saudi Arabian and Mr. Ahmed Hussain Zietoun; Prof. Ahlam Alfazairy (Dept. of Entomology, Faculty of Agric., Univ. of Alexandria), Prof. M. Shaawer (Dept. of Pesticides Chemistry, Faculty of Agric., Univ. of Alexandria), Prof. Dr. Fathallah Zaioon (Dept. of Plant Morphology, Faculty of Agric., Univ. of Alexandria), Prof. Fikry El-Shahawi (Dept. of Pesticides Chemistry, Faculty of Agric., Univ. of Alexandria), Prof. M. Shams (Dept. of Nematology, Faculty of Agric., Univ. of Cairo).

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Metwaly Method Endotherapic Injection for Palm Trees – the New Methods Used to Control the Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier)

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Keywords: *Rhynchophorus ferrugineus*, insecticides, endotherapy

Abstract

The amended Italian tree vital machine, Treevital Endopalm proved its efficiency as a very effective injection machine for palm trees, to control the red palm weevil *Rhynchophorus ferrugineus*, in Italy, Saudi Arabia, and Egypt. Such a patented machine has been used to inject 6 groups of 30 *R. ferrugineus*-infested palm trees (5 trees per group) by 6 insecticides (one/group). The insecticides were as follows: Abamictin, Oxydemethion, Fipronil, Imidacloprid, Azdrachtin and a mix of ten vegetal oils with Zn and Mn.

This method of application was found to be highly economical with less environmental hazards, and safe for the users.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleopetra*: *Curculionidae*) is a very serious pest of cultivated palms in many countries in southern Asia, North Africa, Europe, the Gulf states, and the Middle East. The weevil could invade many other areas and countries where the date palms, the coconut palms, the sago palms, the talipot palms, the oil palms, the royal palms, the sugar, the toddy palms, the serdag palms, the nibong palms ,the areca palms and some other ornamental palms are grown (Van Derlaan, 1981).

Injection of insecticides, to control the palm tree pests, by pipes proved to be not safe for the users with many environmental hazards, time-consuming and not economical. Therefore, the present work was conducted to evaluate one of the patented Italian tree vital machines as an endotherapic method for palm tree treatment.

MATERIALS AND METHODS

Insecticides

The application was carried out testing insecticides in different mix.

The insecticide Oxydemethion showed to be the most virulent one (nearly 98% mortality) but there is no more possibility to employ it.

The insecticides as follows, were tested in different blends: Abamictin, Fipronil, Imidacloprid, Azdrachtin, Biorynk: mix of 10 vegetal oils and Zn+Mn.

All these products were mixed in the recommended concentration and subsequently mixed with plain water to the amount of product depending on the height, diameter and degree of infestation of the palm. According to the formula that determines the method Metwaly the solution dose required for each plant:
(circumference in meters × height in meters×5)/10=product quantity in liters per injection endotherapy on healthy palm
(circumference in meters × height in meters×9)/10=product quantity in liters (for injection endotherapy infested palm).

The application of the tested insecticides for the tested palm trees was carried out

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by adoption of the endotherapic method recommended by Metwaly (2008, 2010) using a new model of his patented Treevital Endopalm Machine (Figs. 1-4) for palm tree-injection. Five palm trees were used per test insecticide, the tested insecticide concentrations ranged from 100 to 200 ml/L distilled water. Injection trials were achieved by an electrical driller (1000 rpm). The palm trees of 35, 50 and 100 cm in diameter were injected.

In order to evaluate the efficiency of both the tested insecticides and the adopted injection new machine (Treevital Endopalm) the following parameters were investigated:
- Effect of the palm tree diameter (35, 50 and 100 cm) on the time required for injection.
- Effect of injection-machine pressure (1, 2, 3, 4, 5 and 6 bar).
- Effect of post-treatment period (3, 7, and 14 days, then 1, 2, 3 and 5 months).

All means were compared using the least significant difference test (LSD) at P=0.05. Also, the completely randomized design with 5 replicates (one palm tree=replicate) was used.

RESULTS AND DISCUSSION

The field observation on the insecticide-treated palm trees, by the endotherapic method using the patented Italian Treevital Endoplam machine to control the red palm weevil, *R. ferrugineus* (Olivier), revealed that all the subject insecticides were efficiently able to control the different stages of the red palm weevil inside the infested palm trees. On the other hand, the insecticide Oxydemethion proved to the most virulent one (nearly 98% mortality) as compared with the insecticide abamictin. 28 palm trees of 30 completely recovered from *R. ferrugineus* infestation (i.e., nearly 95% recovery). Dissection of 5 recovered palm trees showed nearly none of alive stages of the subject insect were observed.

Observations on the effect of the palm tree diameter on the required time for trunk injection indicate that trees with 100 cm in diameter had consumed significantly less time (0.5 min) than that of 35 cm in diameter. Also, when the tree vital injection machine was adjusted at the pressure of 5 bar the required injection time was very short (0.5 s) when compared to the corresponding values at 1, 2, 3 and 4 bar. Pressure of 6 bar was not tested because the first trial showed the vessels damaged.

In addition, the present findings showed that both the adjusted pressure of the subject injection machine and the diameter of the treated palm tree had a significant effect on the required time for injecting the tested insecticides. The diameter of 50 cm and pressure of 5 bar recorded a considerable decrease in the time required for the insecticide injection by such a machine, where only 0.24 s was needed as compared with the corresponding values of 35 cm and 1 bar, respectively.

The advantages of endotherapy performed according to the method with Metwaly Treevital Endopalm are:
- maximum efficiency with minimum doses.
- no environmental impact.
- maximum security for users and operators (both during and after treatment).
- minimum decay of the product and resulting in greater period of efficiency. The product is protected from degradation of the weather, this is especially true for the active ingredients of biological origin.
- increase the effectiveness of organic products that interact with the immune system of the plant.
- careful control of pressure to fit the physiological state of the specimen to be treated.
- ability to work ‘continuously’ to avoid air gaps and breaks in administration.
- definition of the correct amount of solution to be administered by the formula adopted by Metwaly and the meter automatically.
- simple operation and small footprint of the machine for operations ‘share in’.
- needles and other accessories allow to reach all organs of the plant (roots to the capital for any size of plant).
- the materials are of high quality and are designed to operate in adverse conditions.

Previous experiments on 3500, 600 and 5000 palm trees in Italy, Saudi Arabia, and Egypt, respectively, using the same Italian Treevital Endopalm machine to control the red palm weevil and other insect pests of palm trees, could confirm the present findings and may emphasize the promising role of a such endotherapic method for the palm tree by using the Treevital Endopalm machine.

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Figures

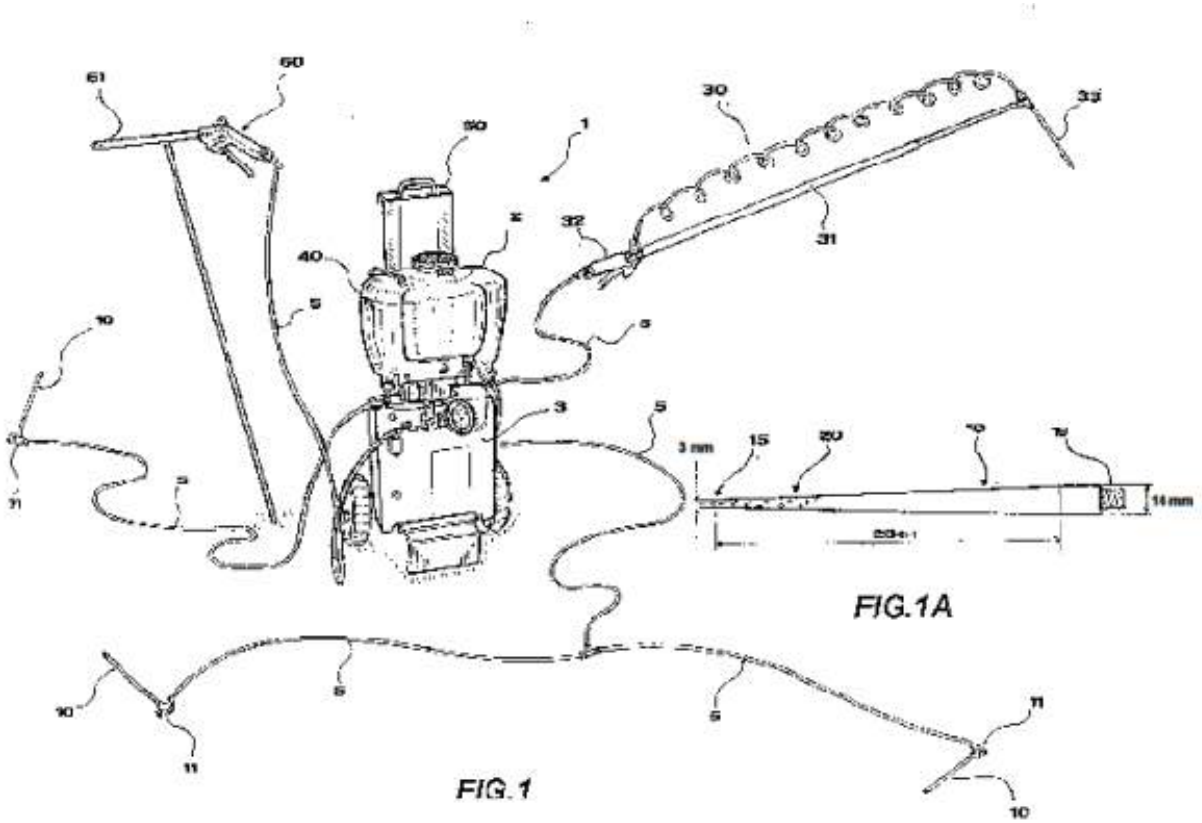

Fig. 1. Treevital Endopalm machine.

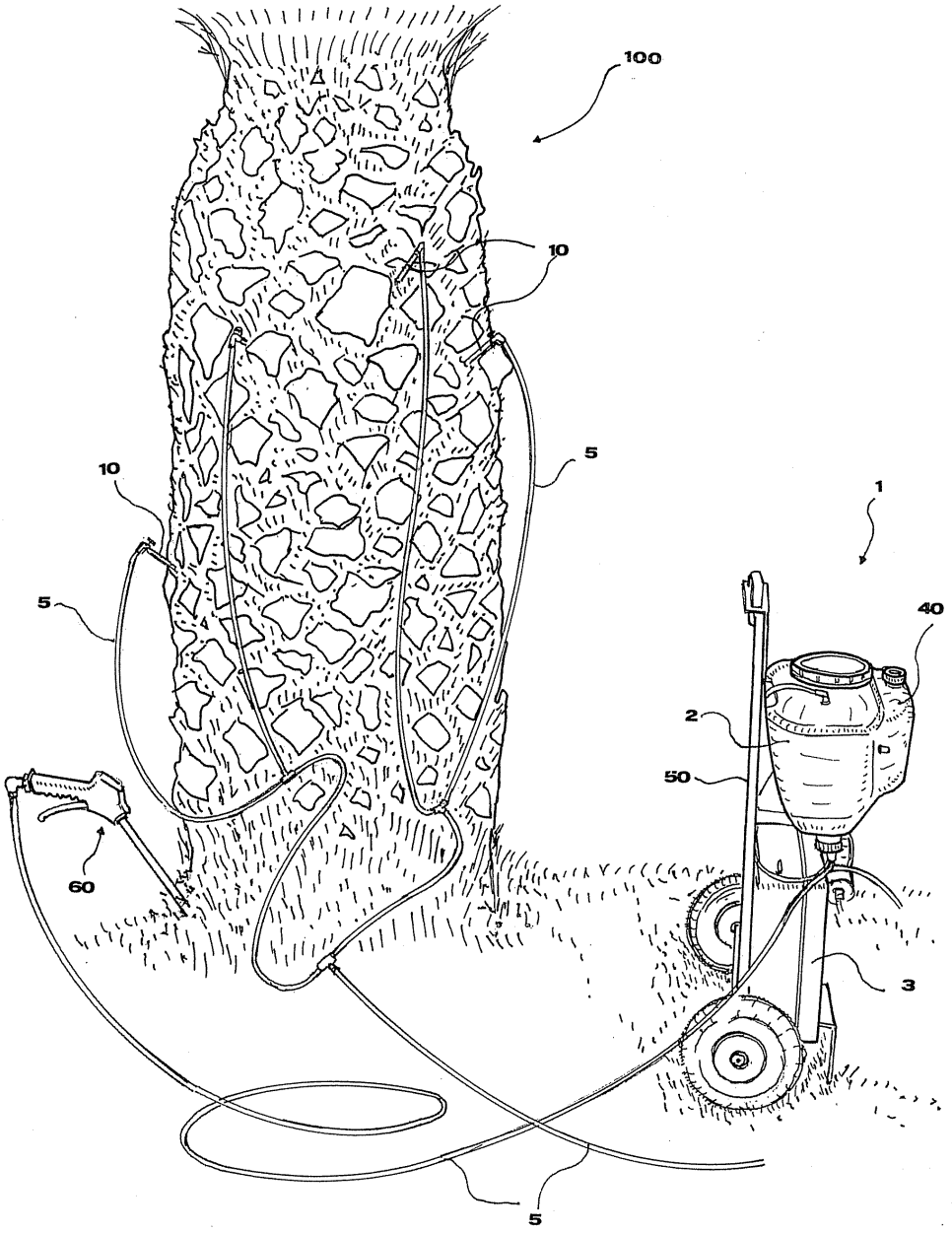

Fig. 2. Treevital Endopalm machine.

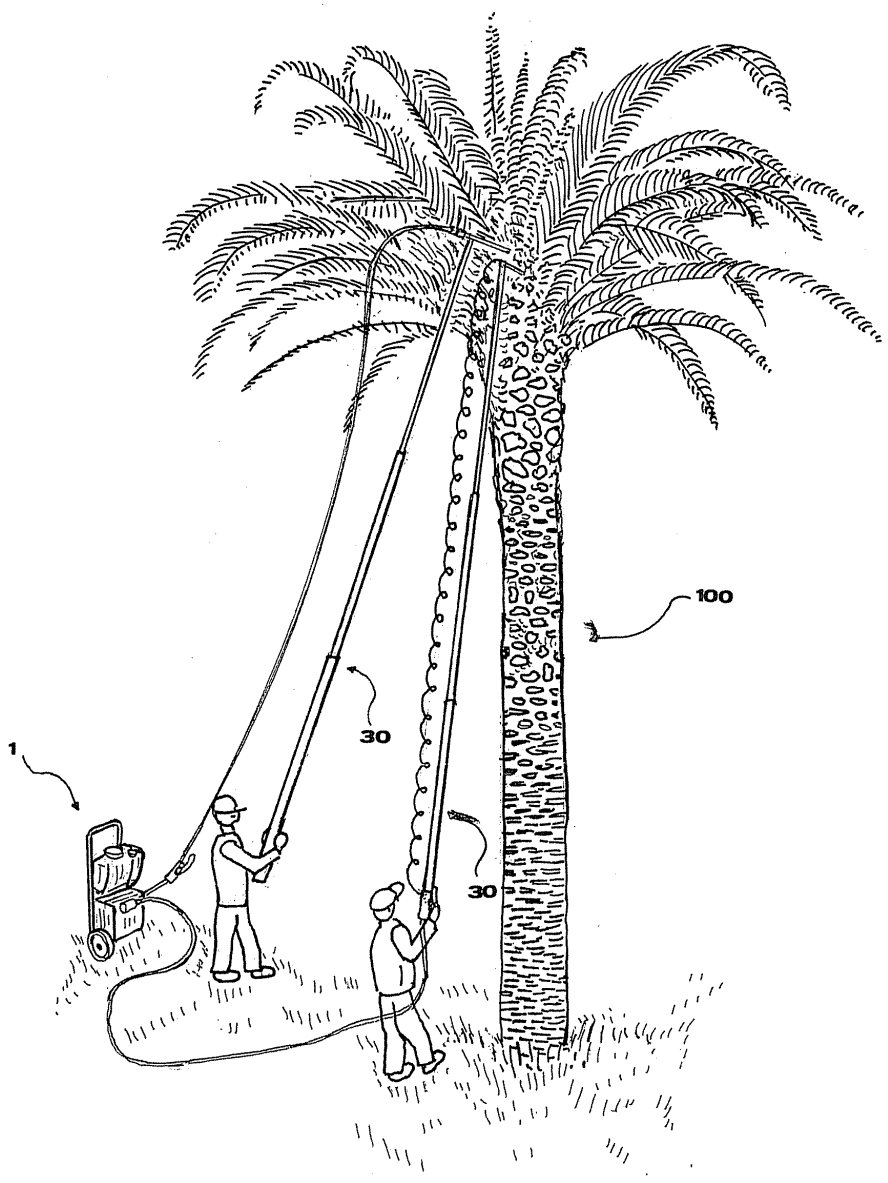

Fig. 3. Treevital Endopalm machine.

Fig. 4. Front of the Treevital Endopalm machine. 1. Plastic Handle (COD 20012); 2. Tank cap handle (COD 20013); 3. Main tank cap (COD 20014); 4. Container for clean water basin (COD 20015); 5. Container principal in plastic 13-L scale (COD 20016); 6. Basin faucet tank (COD 20018); 7. Mixing liquid (COD 20019); 8. Indicator battery charge led (COD 20020); 9. Vacuum tube product (COD 20021); 10. Product mixing tube (COD 20022); 11. Control unit pressure with external gauge (COD 20023); 12. Darin to product recovery (COD 20024); 13. Delivery valve (COD 20025); 14. Gauge (COD 20026); 15. Box system (COD 20027); 16. Rubber wheels (COD 20028); 17. Steel frame (COD 20028); 18. Box for objects in plastic (COD 20029).

Surveying *Ephestia* spp. and the Parasitoid *Bracon hebetor* in Date Palm Orchards and Date Stores of Five Governorates in Iraq

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Abstract

The results are presented of the survey for *Ephestia* spp. and the parasitoid *Bracon hebetor* in date stores and in date palm orchards of five governorates (Baghdad, Karbala, Najaf, Babylon and Al-Qadesyia). These orchards are planted with the following cultivars of date palms: 'Zahdi', 'Sayer', 'Helawi' and 'Khadrawi', these four cultivars represent 76% of date trade in Iraq. It was revealed that the percentages of infested dates with *Ephestia* spp. larvae were 1.4, 16.5 and 14.4% in the dates collected either directly from date palms or collected from under the trees (fallen date) or from date stores in the Baghdad governorate. These percentages were 2.1, 11.0 and 22.7% for Karbala governorate, 2.6, 20.4 and 17.5% for Najaf governorate, 3.3, 14.1 and 27.7% for Babylon governorate and finally 0.9, 7.6 and 9.5% for Al-Qadesyia governorate. Furthermore the results showed that the percentage of paralyzed *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* in the dates collected directly from the date palm trees, in the dates fallen under the date palm trees and in the dates examined from date stores were 22.7, 40.2 and 31.1% for Baghdad governorate, 47.6, 68.0 and 49.0% for Karbala governorate, 16.7, 53.3 and 30.2% for Najaf governorate, 40.0, 36.8 and 20.8% for Babylon governorate and 42.8, 75.0 and 67.4% for Al-Qadeyia governorate.

These results suggest that the parasitoid *Bracon hebetor* accompanied its host *Ephestia* spp. larvae anywhere and it is possible to use it for controlling *Ephestia* spp. larvae instead of methyl bromide within an IPM project to control *Ephestia* spp. in Iraq.

INTRODUCTION

It is believed that the main problem facing date trade in Iraq is its infestation with insect pests, specifically the ones belonging to the *Lepidoptera* order because their larvae feed upon date fruit in the field and in the store (Al-Baker, 1972; Al-Haidari, 1979; Al-Hussain, 1985; Khadir, 1998). If this infestation is left without any treatment, the dates will be seriously damaged and become unsuitable for human consumption (Al-Baker, 1972; Al-Hafith et al., 1987; Al-Haidari, 1979; Al-Husain, 1985). Therefore, to control this infestation with pesticide, it appeared that methyl bromide (CH_3Br) was the only fumigant to be used for date fumigation to kill most insect stages because of its ability to penetrate through date boxes (Al-Hafith et al., 1987; Al-Haidari, 1979; Al-Hussain, 1985). But using this fumigant in the meantime is facing several problems such as a) developing resistance in the target insect pest and in our case *Ephestia* spp., b) contaminating the environment and it appeared to be a carcinogenic agent to human beings, c) it is an unspecific agent killing useful and harmful insect species and d) it appeared to be an ozone depleting agent (Ahmed, 1998; Al-Haidari, 1979; Al-Hussain, 1985; Lindgren, 1968). For all these reasons a decision was taken during the Montreal Meeting to stop using it in near future (Ahmed, 1998; Al-Taweel and Al-Jboory, 2007; Abdullah and Sabih, 1999; Marcotte, 1993; Ross and Vail, 1993). Moreover, Iraq signed

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the Montreal Protocol during the recent meeting of West Asian Office/UN which was held on 24-25 May 2008 in Cario, Egypt, on the alternative materials to methyl bromide in disinfesting dates during storage. In this respect it is important to mention that Iraq started to look for alternative methods for controlling *Ephestia* spp. in dates because dates are very important for its trade since the seventies of the last century (Ahmed, 1998; Al-Taweel and Al-Jboori, 2007; Khadir, 1998; Ahmed et al., 1972; Al-Taweel et al., 1995) because Iraq produces more than 500 thousand tons of dates annually (Annual Statistical Abstract, 2006). One of these methods is the biological control using *Bracon hebetor* (Al-Taweel and Al-Jboori, 2007; Hameed et al., 2004, 2005, 1999; Hameed, 2002) as a parasitoid to control larvae of target insect pest (*Ephestia* spp. larvae), but before using this method as an alternative to methyl bromide for controlling *Ephestia* spp. larvae in dates, it is important to carry out a survey in the fields and stores to know the rate of infestation of dates by *Ephestia* spp. larvae and the presence of *Bracon hebetor* naturally in the fields because this will give us an idea about the number needed from this parasitoid to be produced for releasing in the future. This investigation will concentrate upon these aims.

MATERIALS AND METHODS

Field Survey for *Ephestia* spp. Larvae and the Parasitoid *B. hebetor* in the Dates Left on the Date Palm Trees without Collection

Five orchards were selected for this study, the area of each orchard was 2-7.5 ha, each orchard was located in one of the following governorates: Baghdad, Karbala, Najaf, Babylon and Al-Qadesyia. These orchards are planted with the following date palm tree cultivars, 'Zahdi', 'Sayer', 'Helawi' and 'Khadrawi'. Each orchard was divided into five sections and ten date palm trees were selected randomly from each section of each orchard for each governorate mentioned above. These date palm trees were of different heights, dates were collected directly from these date palm trees and kept into polyethylene bags and brought to the laboratory for examination.

Field Survey for *Ephestia* spp. Larvae and the Parasitoid *B. hebetor* in the Dates Collected from under the Date Palm Trees (Fallen Dates)

The fallen dates were collected from under the date palm trees randomly from each orchard selected for this study as mentioned in paragraph one above and kept into polyethylene bags and were brought to the laboratory for examination.

Survey for *Ephestia* spp. Larvae and the Parasitoid *B. hebetor* in the Dates Collected from Date Stores

The survey was carried out in the date stores located in the governorates mentioned in the first paragraph above. It is interesting to mention that dates were stored in the date stores either in plastic boxes in rows or directly on the floor of the stores. In both cases the height of the dates that could be reached was 6 m. Again the stores were divided into five sections, the date samples were collected from each section from two levels (top and medium) and kept into polyethylene bags and were brought to the laboratory for examination.

RESULTS AND DISCUSSION

The results are illustrated in Tables 1, 2, 3, 4 and 5 for the governorates Baghdad, Karbala, Najaf, Babylon and Al-Qadesyia and show that the percentage of infested dates with live larvae of *Ephestia* spp. collected directly from the date palm trees were 1.4, 2.1, 2.8, 3.3 and 0.9% respectively, while for that collected from under the date palm trees (fallen dates) these were 16.5, 11.0, 20.4, 14.1 and 7.6% for the same governorates respectively. Finally, the percentage of infested dates with *Ephestia* spp. larvae in the dates collected from date stores were 14.4, 22.7, 17.5, 27.7 and 9.5% for the same governorate respectively. Furthermore, the results of the same tables show that the

numbers of live larvae of *Ephestia* spp. were 22, 42, 12, 10 and 7 larvae in the dates collected from date palm trees directly, while 82, 153, 30, 38 and 16 larvae in the dates collected from under the date palm trees (fallen dates) and 61, 100, 106, 72 and 43 larvae in the dates examined from date stores for the same governorates, respectively. From these results it is clearly seen that *Ephestia* spp. infests dates either on the date palm trees or fallen dates on the floor of the orchards. Moreover, this infestation will develop in the stores as a results of suitable conditions (temperature and relative humidity). Therefore, *Ephestia* spp. appeared to infest dates in the field and then this infestation will increase in the date stores before processing dates (Al-Taweel and Al-Jboori, 2007; Hussain and Esmael, 2007; Al-Taweel et al., 1995). Moreover, it was noticed from the results demonstrated in the same Tables 1, 2, 3, 4 and 5 that the percentages of paralyzed *Ephestia* spp. larvae by *B. hebetor* that were found in the dates collected directly from the date palm trees were 22.7, 47.6, 16.7, 40.0 and 42.8%, while it was 40.1, 68.0, 53.3, 36.8 and 75.0% in the fallen dates and it was 31.1, 49.0, 30.2, 20.8 and 67.4% in the dates collected from date stores for the governorates under investigation. Again these results show that the parasitoid *B. hebetor* accompanied its host anywhere. This means that it was found in the dates collected directly from the date palm trees, dates fallen under the date palm trees and dates in the date stores. Finally the results of Tables 1, 2, 3, 4 and 5 revealed that the parasitoid *B. hebetor* was found in most of the 2009 months. These results illustrate the efficacy of this parasitoid in controlling *Ephestia* spp. larvae which resulted in reducing the population of *Ephestia* spp. (Hameed et al., 2004, 2005; Hameed, 2002).

In conclusion *B. hebetor* accompanied its host *Ephestia* spp. larvae in the fields and stores. This shows the possibility in using it as a biological element within an IPM project to control *Ephestia* spp. larvae in dates before processing them for human consumption.

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Tables

Table 1. Number of examined dates, number of infested dates collected directly from date palm trees, fallen dates and dates collected from date store and the percentage of paralized *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* during 2009 in Baghdad.

Type of examination		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%	Average	Notes
No. of examined dates	On date palm trees	215	380	451	616	320	416	553	390	280	850	911	790	6172			
	No. of infested dates	6	5	9	6	7	5	8	10	14	6	4	5	85			
	% infestation	2.8	1.3	2.0	2.0	2.2	1.2	1.4	2.6	5.0	0.7	0.4	0.6		1.4		
No. of examined dates	Fallen under the trees	610	528	915	783	650	800	517	480	753	900	530	491	7957			
	No. of infested dates	118	96	75	111	122	65	81	51	507	36	18	32	1312			
	% infestation	19.3	18.2	8.2	14.2	18.8	8.1	15.6	10.6	67.3	4.0	3.4	6.5		16.5		
No. of examined dates	From date store	450	800	750	620	530	638	530	459	596	-	731	820	6924			
	No. of infested dates	27	56	240	51	112	125	128	134	84		17	25	999			
	% infestation	6.0	7.0	32.0	8.2	21.1	19.6	24.2	29.2	14.1	-	2.3	3.0		14.4		
No. of live *Ephestia* spp. larvae	In date collected from date palm trees	0	1	2	1	5	3	4	1	0	2	1	2	22		1.8	
	In fallen dates	2	2	8	9	5	11	4	6	4	10	14	7	82		6.8	
	In dates collected from date stores	8	5	4	1	3	2	12	15	7	-	3	1	61		5.1	
No. of paralized *Ephestia* spp. larvae	In date collected from date palm trees	0	0	0	1	2	1	0	0	0	1	0	0	5	22.7		
	In fallen dates	0	0	4	6	4	3	2	1	2	3	4	4	33	40.2		
	In dates collected from date stores	0	1	2	4	2	1	1	3	4	-	1	0	19	31.1		
No. of *B. hebetor* stages* found	In date collected from date palm trees	0	0	1	1	0	0	0	0	1	1	1	0	5		0.4	
	In fallen dates	0	1	0	0	1	0	1	0	0	0	0	1	4		0.3	
	In dates collected from date stores	2	1	1	3	2	1	1	2	3	-	6	2	24		2.0	

* *B. hebetor* larvae, pupae, adults, - Means no dates in date store.

Table 2. Number of examined dates, number of infested dates collected directly from date palm trees, fallen dates and dates collected from date store and the percentage of paralized *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* during 2009 in Karbala.

Type of examination		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%	Average	Notes
No. of examined dates	On date palm trees	170	241	301	411	375	586	711	386	290	401	250	280	4422			
	No. of infested dates	4	18	11	12	9	5	12	6	6	5	2	3	93			
	% infestation	2.4	7.5	3.7	2.9	2.4	0.8	1.7	1.6	2.1	1.2	0.8	1.1		2.1		
No. of examined dates	Fallen under the trees	450	390	461	629	448	246	318	606	357	780	800	610	6101			
	No. of infested dates	64	48	62	72	76	49	55	127	57	10	18	34	672			
	% infestation	14.2	12.3	13.4	11.4	16.9	19.9	17.3	20.9	16.0	1.3	2.3	5.6		11.0		
No. of examined dates	From date store	497	632	700	2469	1500	560	741	480	280	-	1457	670	9986			
	No. of infested dates	40	128	210	565	391	227	262	226	174	-	27	15	2265			
	% infestation	8.0	2.5	30.0	22.9	26.1	40.5	35.4	47.1	62.1	-	1.6	2.2		22.7		
No. of live *Ephestia* spp. Larvae	In date collected from date palm trees	3	2	4	5	6	4	1	0	4	6	5	2	42		3.5	
	In fallen dates	4	6	10	22	33	18	5	3	11	14	18	9	153		12.8	
	In dates collected from date stores	8	5	14	9	13	12	10	15	7	-	3	4	100		8.3	
No. of paralized *Ephestia* spp. larvae	In date collected from date palm trees	1	0	3	2	4	2	0	0	3	2	2	1	20	47.6		
	In fallen dates	2	3	7	13	26	10	8	6	5	10	9	5	104	68.0		
	In dates collected from date stores	5	3	8	5	6	7	4	6	3	-	0	2	49	49.0	49.0	
No. of *B. hebetor* stages* found	In date collected from date palm trees	0	1	1	2	1	2	1	1	0	1	1	1	12		1.0	
	In fallen dates	2	4	7	15	23	13	6	4	8	11	16	6	115		9.6	
	In dates collected from date stores	3	5	4	6	3	2	1	2	4	-	7	4	41		3.4	

* *B. hebetor* larvae, pupae, adults, - Means no dates in date store.

Table 3. Number of examined dates, number of infested dates collected directly from date palm trees, fallen dates and dates collected from date store and the percentage of paralized *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* during 2009 in Najaf.

Type of examination		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%	Average	Notes
No. of examined dates	On date palm trees	243	151	117	242	157	117	240	218	192	390	405	617	3089			
	No. of infested dates	5	8	6	10	4	4	6	9	10	9	3	6	80			
	% infestation	2.1	5.3	5.1	4.1	2.5	3.4	2.5	4.1	5.2	2.3	0.7	1.0		2.6		
No. of examined dates	Fallen under the trees	511	602	396	453	772	552	630	380	411	820	700	643	6870			
	No. of infested dates	100	162	136	198	125	118	144	215	130	15	23	35	1401			
	% infestation	19.7	27.0	34.3	43.7	16.2	21.4	22.9	56.6	31.6	1.8	3.3	5.4		20.4		
No. of examined dates	From date store	650	490	555	564	637	830	750	420	381	-	913	910	6600			
	No. of infested dates	54	45	107	149	143	181	175	138	124	-	13	26	1155			
	% infestation	8.3	9.2	19.3	26.4	22.4	21.8	23.3	32.2	32.5	-	1.4	2.9		17.5		
No. of live *Ephestia* spp. larvae	In date collected from date palm trees	0	1	0	4	3	0	0	2	1	0	0	1	12		0.6	
	In fallen dates	2	1	3	5	2	1	3	2	4	1	2	4	30		2.5	
	In dates collected from date stores	6	8	13	19	17	5	8	6	12	-	4	8	106		8.8	
No. of paralized *Ephestia* spp. larvae	In date collected from date palm trees	0	0	0	1	1	0	0	0	0	0	0	0	2	16.7		
	In fallen dates	1	0	2	4	1	0	2	1	3	0	0	2	16	53.3		
	In dates collected from date stores	0	2	4	5	9	2	1	2	2	-	2	3	32	30.2		
No. of *B. hebetor* stages* found	In date collected from date palm trees	0	0	1	2	3	0	0	1	1	1	1	1	11		0.9	
	In fallen dates	2	1	2	3	1	2	0	0	1	1	2	1	16		1.3	
	In dates collected from date stores	4	5	2	3	7	3	1	2	3	-	2	5	37		3.1	

* *B. hebetor* larvae, pupae, adults, - Means no dates in date store.

Table 4. Number of examined dates, number of infested dates collected directly from date palm trees, fallen dates and dates collected from date store and the percentage of paralized *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* during 2009 in Babylon.

Type of examination		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%	Average	Notes
No. of examined dates	On date palm trees	180	270	231	500	560	300	319	209	189	700	590	841	4889			
	No. of infested dates	5	10	11	28	34	12	9	3	14	9	12	15	162			
	% infestation	2.8	3.7	4.8	5.6	6.1	4.0	2.8	1.4	7.4	1.3	2.0	1.8		3.3		
No. of examined dates	Fallen under the trees	350	620	500	412	390	642	518	492	405	618	982	560	6489			
	No. of infested dates	72	139	74	67	94	118	129	91	68	12	28	21	913			
	% infestation	20.6	22.4	14.8	16.3	24.1	18.4	24.9	18.5	16.8	1.9	2.8	3.8		14.1		
No. of examined dates	From date store	466	700	802	513	499	361	419	650	508	-	1500	820	7238			
	No. of infested dates	98	167	150	143	199	172	201	445	390	-	24	17	2006			
	% infestation	21.0	23.8	18.7	27.9	39.8	47.6	48.0	68.0	76.0	--	1.6	2.1		27.7		
No. of live *Ephestia* spp. larvae	In date collected from date palm trees	1	0	1	2	2	1	0	0	0	2	1	0	10		0.8	
	In fallen dates	2	1	2	4	6	5	4	3	5	0	2	4	38		3.2	
	In dates collected from date stores	6	5	3	15	13	8	4	3	9	-	2	4	72		6.0	
No. of paralized *Ephestia* spp. larvae	In date collected from date palm trees	0	0	1	1	1	0	0	0	0	1	0	0	4	40.0		
	In fallen dates	1	0	0	3	2	2	1	0	2	0	1	2	14	36.8		
	In dates collected from date stores	2	0	0	4	1	3	2	0	2	-	0	1	15	20.8		
No. of *B. hebetor* stages* found	In date collected from date palm trees	0	1	0	1	1	0	2	0	1	1	0	2	9		0.8	
	In fallen dates	2	1	1	6	3	2	1	1	2	1	2	1	23		1.9	
	In dates collected from date stores	3	6	5	9	6	2	5	6	8	-	9	5	64		5.3	

* *B. hebetor* larvae, pupae, adults, - Means no dates in date store.

Table 5. Number of examined dates, number of infested dates collected directly from date palm trees, fallen dates and dates collected from date store and the percentage of paralized *Ephestia* spp. larvae by the parasitoid *Bracon hebetor* during 2009 in Al-Qadesyia.

Type of examination		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%	Average	Notes
No. of examined dates	On date palm trees	120	203	190	311	200	130	415	318	121	2500	1300	850	6658			
	No. of infested dates	2	3	4	2	2	2	4	5	3	12	11	12	62			
	% infestation	1.7	1.8	2.1	0.6	1.0	1.5	0.9	1.6	2.5	0.5	0.8	1.4		0.9		
No. of examined dates	Fallen under the trees	218	360	391	440	521	319	220	326	210	590	740	613	4948			
	No. of infested dates	11	29	26	48	61	21	25	52	35	13	25	31	377			
	% infestation	5.0	8.0	66.7	11.0	11.7	6.6	11.4	16.0	21.7	2.2	3.4	5.0		7.6		
No. of examined dates	From date store	500	620	751	800	410	450	380	515	240	-	850	729	6245			
	No. of infested dates	22	19	42	51	61	70	79	105	100	-	19	25	593			
	% infestation	4.4	3.1	5.6	6.8	14.8	15.6	20.8	20.4	41.6	-	2.2	3.4		9.5		
No. of live *Ephestia* spp. larvae	In date collected from date palm trees	0	0	1	1	0	0	1	0	1	1	1	1	7		0.6	
	In fallen dates	1	2	3	0	1	3	1	1	0	1	2	1	16		1.3	
	In dates collected from date stores	4	2	5	4	7	3	8	2	2	--	1	5	43		3.6	
No. of paralized *Ephestia* spp. larvae	In date collected from date palm trees	0	0	1	1	0	0	1	0	0	0	0	0	3	42.8		
	In fallen dates	1	1	2	1	1	2	0	1	0	1	2	0	12	75.0		
	In dates collected from date stores	2	2	3	5	7	1	4	2	1	-	0	2	29	67.4		
No. of *B. hebetor* stages* found	In date collected from date palm trees	1	1	5	12	1	1	3	0	1	4	2	1	32		2.7	
	In fallen dates	8	3	5	2	6	1	3	4	9	0	0	2	43		3.6	
	In dates collected from date stores	2	8	6	15	8	4	7	2	5	-	2	9	68		5.7	

* *B. hebetor* larvae, pupae, adults, - Means no dates in date store.

Control of the Greater Date Moth, *Arenipses sabella* Hampson (*Lepidoptera*: *Pyralidae*) Using Biopesticides

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Keywords: date palm, greater date moth, *Arenipses sabella*, biopesticides, biological control

Abstract

Two seasons of field trials were conducted to investigate the efficacy of certain insecticides and bio-insecticides against the greater date moth, *Arenipses sabella* Hampson (*Lepidoptera*: *Pyralidae*) at El-Kharga oasis, New Valley, Egypt in 2006/2007and 2007/2008. The high effects against *A. sabella* among the tested compounds were recorded in case of the treatment of date palm with Proclaim 05 SG (20 g/100 L water) and Deltachem super 2.6 EC (50 ml/100 L water) during the two seasons. The treatment with Proclaim twice during 1 and 22 November of 2006 reduced the rate of bunches infestation by the pest to 85.48% in the 2007 season. During 2008 season, application with Deltachem super during 1 and 22 November of 2007 reduced the level of infestation by the greater date moth pest to 75.74%.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is considered one of the most important cash crops in New Valley Governorate. In this Governorate more than one million date palm trees are grown. Besides the local consumption, dates are also exported to foreign countries.

The lesser date moth *Batrachedra amydraula* Meyrick, the pomegranate, *Viracola livia* Klug and the almond moth *Cadra* spp. are considered the most economically important insect pests attacking date palm trees in this area (Saleh, 1974; Temerak and Sayed, 1995; Sayed and El-Deeb, 1996; Sayed et al., 2001).

Arenipses sabella (*Lepidoptera*: *Pyralidae*) is an early season pest. Spathes, bunches and fruit stalks were attacked in March and early April, and infestation was high at the end of April. When the larvae infested at the later stage of growth, bunch bases broke and caused superficial damage to fruits and affected its quality. This usually happens during August when such bunches are heavy enough and then these infested bunches are unable to bear their weight. Two generations per year were recorded (Saleh, 1974; Ali et al., 1988; Abdel-Rahman et al., 2007).

Recently, *A. sabella* became a major pest attacking the date palm trees in the New Valley. Little information was available in the literature concerning to control of *A. sabella*. Therefore, the present study was conducted to evaluate certain insecticides and bio-insecticides against this pest.

MATERIALS AND METHODS

Trials were conducted in Ganah Village, Kharga Oasis in 2006/2007 and 2007/2008. The following treatments were: 1. Tracer 24% SC (Spinosad) containing 240 active ingredient as spinosad (Spynosin A&D). It is a natural metabolite of the Actinomycete, *Aaccharopolyspora spinosa* Mertz & Yao; 2. Radiant 12SC (Spinetoram) containing 120 active ingredient as spinosad (Spinosyn J&L). It is derived from fermentation of the Actinomycete, *S. spinoza*; 3. Runner 24% SC (Methoxyfenozide); 4. Deltachem 2.6 EC (Deltamethrin 2.6%); 5. Match 5%ES (Lufenuron); 6. Proclaim 05 SG (Emamectin benzoate).

The time of applications and the compound rates are recorded in Table 1. Date palm cultivar 'Saidi' ('Sewi') was used. All treatments were replicated four times. One date palm tree was considered as one replicate. Samples size was all bunches/one date

palm. Inspection times were conducted at two weeks interval from the middle of April until 15 May during the two successive seasons (2007 and 2008).

RESULTS AND DISCUSSION

Data in Table 2 indicate that all tested compounds induced a remarkable reduction on the infestation rates with the greater date moth with different levels during the season of 2007.

The results indicate that the low effects against *A. sabella* (17.45, 30.13, 37.50, 37.84 and 38.63 %), were recorded in case of the treatment with one spray of Match (40 ml), Tracer/Runner (20/20 ml), Radiant/Runner (10/20 ml), one spray of Proclaim (20 g) and Tracer/Runner (20/25 ml).

The highest reduction in the date palm bunches infestation with no significant differences (85.82, 85.45 and 85.19%) were obtained with Proclaim 20 g (2, 3 and 4 sprays), respectively.

Data presented in Table 3 show the percentage in reduction of the date palm bunches infestation due to treatment with the tested compounds during 2008 season.

In general, the treatments with Radiant/Runner or Tracer/Runner induced the lowest reduction percentages of the infestation with the greater date moth.

Treatments with Deltachem 40 ml (2 and 4 sprays) recorded the highest reduction percentages infestation to the date palm bunches with no significant differences (75.74 and 75.25%), respectively. On the other hand, treatments with Proclaim 20 g (2, 3 and 4 sprays) gave good results with no significant differences recorded (73.43, 72.83 and 73.02%), respectively.

The present results indicate that bio-insecticides like Tracer and Radiant when applied as a rotation program with Runner gave the lowest effects against *A. sabella* followed by Match compound. Meanwhile, the highest reduction percentages of the infestation with the greater date moth were obtained when the date palm was treated with Proclaim 20 g or Deltachem 40 ml two sprays during 1 and 22 November.

Treatment of the date palms during 1 and 22 November with Proclaim or Deltachem may reduce the population density of *A. sabella* larvae which will enter as overwintering larvae or pupae (Abdel-Rahman et al., 2007). Then the population density of the emergence adults will be low during the spring season.

It could be useful to recommend the use of Proclaim 20 g or Deltachem 40 ml, 2 sprays during 1 and 22 November.

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Tables

Table 1. Treatments, rates and time of applications on *Arenipses sabella*.

No.	Treatments and rates/100 L water			
	1/11	22/11	13/12	3/1
1	Radiant 10 ml	Runner 20 ml	------	
2	Radiant 10 ml	Runner 25 ml	------	-----
3	Radiant 20 ml	Runner 20 ml	------	-----
4	Radiant 20 ml	Runner 25 ml	------	-----
5	Tracer 20 ml	Runner 20 ml	------	-----
6	Tracer 20 ml	Runner 25 ml	------	-----
7	Tracer 30 ml	Runner 20 ml	------	-----
8	Tracer 30 ml	Runner 25 ml	------	-----
9	Deltachem 50 ml	------	------	-----
10	Deltachem 50 ml	Deltachem 50 ml	------	-----
11	Deltachem 50 ml	Deltachem 50 ml	Deltachem 50 ml	Deltachem 50 ml
12	Deltachem 50 ml	Deltachem 50 ml	Deltachem 50 ml	----
13	Match 40 ml	-----	-----	-----
14	Match 40 ml	Match 40 ml	-----	-----
15	Match 40 ml	Match 40 ml	Match 40 ml	Match 40 ml
16	Match 40 ml	Match 40 ml	Match 40 ml	-----
17	Proclaim 20 g	-----	-----	-----
18	Proclaim 20 g	Proclaim 20 g	-----	-----
19	Proclaim 20 g	Proclaim 20 g	Proclaim 20 g	Proclaim 20 g
20	Proclaim 20 g	Proclaim 20 g	Proclaim 20 g	

Statistical analysis was done for infestation figures which turned then after to reduction % based on Abbott formula (1925). Data were statistically analyzed by F-test and the means were compared according to Duncan's Multiple Range Test (Snedecor and Cochran, 1971).

Table 2. Percent reduction of *Arenipses sabella* infestation of date palm bunches New Valley, 2007.

Treatment	Rate (ml/ 100 L)	Sampling dates						Mean
		15/4		1/5		15/5		
		Infes.	Red.	Infes.	Red.	Infes.	Red.	
Radiant/Runner	10 and 20	16.67	49.98	38.89	30.29	38.89	32.24	37.50 I
	10 and 25	11.11	66.66	33.30	40.00	33.33	41.93	49.53 G
	20 and 20	10.81	67.56	27.02	51.34	27.02	52.92	57.27 F
	20 and 25	8.89	73.32	25.00	54.99	25.00	56.44	61.58 DE
Tracer/Runner	20 and 20	20.83	37.50	33.33	40.00	50.00	12.89	30.13 J
	20 and 25	20.40	38.79	34.69	37.55	34.69	39.56	38.63 I
	30 and 20	18.38	44.85	18.38	66.91	18.38	67.97	59.91 E
	30 and 25	17.07	48.78	19.51	64.87	19.51	66.01	59.88 E
Deltachem								
One spray	50	19.05	42.88	36.51	34.27	36.51	36.39	37.84 I
Two sprays		9.37	71.88	21.87	60.62	21.87	61.89	64.79 C
Three sprays		9.61	71.15	23.07	58.45	23.07	59.80	63.13 CD
Four sprays		9.30	72.00	23.26	58.12	23.26	59.47	63.19 CD
Match								
One spray	40	28.57	14.28	45.71	17.71	45.71	20.36	17.45 K
Two sprays		20.00	39.99	32.00	42.39	32.00	44.25	42.21 H
Three sprays		18.46	44.61	33.33	39.99	33.33	41.93	42.17 H
Four sprays		10.44	68.67	16.11	70.99	20.89	63.59	67.75 B
Proclaim								
One spray	20 g	14.29	57.12	22.22	60.00	25.39	55.76	57.62 F
Two sprays		4.00	87.99	8.33	84.99	8.33	85.48	85.82 A
Three sprays		3.57	87.99	8.92	83.92	8.92	84.45	85.45 A
Four sprays		5.00	84.99	6.66	88.01	10.00	82.57	85.19 A
Control		33.33		55.55		57.40		

Means within columns followed by the same letter(s) are not significantly different at 0.05 level of probability.

Table 3. Percent reduction of *Arenipses sabella* infestation of date palm bunches New Valley, 2008.

Treatment	Rate (ml/ 100 L)	Sampling dates						Mean
		15/4		1/5		15/5		
		Infes.	Red.	Infes.	Red.	Infes.	Red.	
Radiant/Runner	10 and 20	47.16	13.75	54.18	0.23	54.71	5.36	6.44 M
	10 and 25	36.95	32.42	54.34	0.62	54.34	6.00	13.01 L
	20 and 20	31.91	41.64	48.93	10.51	48.93	15.36	22.50 K
	20 and 25	29.51	46.03	37.70	31.05	37.7	34.78	37.28 I
Tracer and Runner	20 and 20	39.28	28.16	44.64	18.36	44.64	22.78	23.10 K
	20 and 25	25.00	54.27	40.06	26.73	40.06	30.70	37.23 I
	30 and 20	22.06	59.65	38.23	30.08	38.23	33.86	41.19 G
	30 and 25	20.63	62.27	36.51	33.22	36.51	36.84	44.11 F
Deltachem								
One sprays		25.49	53.38	41.17	24.70	41.17	28.78	35.62 J
Two sprays	50	12.50	77.13	12.5	77.13	15.62	72.98	75.74 A
Three sprays		10.00	81.71	17.50	67.99	17.50	69.72	73.14 B
Four sprays		12.82	76.55	12.82	76.55	15.38	72.66	75.25 A
Match								
One sprays		31.11	43.10	33.33	39.04	35.55	38.50	40.21 H
Two sprays	40	25.81	52.79	25.81	52.75	28.12	51.35	52.29 E
Three sprays		25.37	53.60	25.37	53.60	28.86	50.07	52.42 E
Four sprays		21.54	60.60	24.61	54.99	24.61	57.42	57.67 D
Proclaim								
One sprays		19.35	64.61	22.25	59.30	22.25	61.51	61.80 C
Two sprays	20 g	14.28	73.88	14.28	73.88	15.87	72.54	73.43 B
Three sprays		12.28	77.54	14.03	74.34	19.29	66.63	72.83 B
Four sprays		13.20	75.85	13.20	75.85	18.86	67.37	73.02 B
Control		54.68		54.68		57.81		

Means within columns followed by the same letter(s) are not significantly different at 0.05 level of probability.

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Biocides, Soil Solarization and Fumigation to Control *Fusarium oxysporum* f. sp. *albedinis* Inciting Bayoud Disease on Date Palm

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Keywords: Bayoud, solar heating, metam sodium, shrimps shell, vetiver

Abstract

In quest of developing an effective strategy to control *Fusarium oxysporum* f. sp. *albedinis,* inciting Bayoud disease on date palm in morocco, the effects of soil solarization and metam sodium, alone or in combination, on survival of total fungi and *Fusarium* spp. were investigated in open field. At 0-20 cm depth, all treatments were almost 100% effective. At 20-40 cm depth, they eliminated more than 80% of total fungi and 90% of *Fusarium* spp. At 40-60 cm depth, a combination of reduced doses of metam sodium and soil solarization was more effective. Also, many natural products were tested for their effect on *Fusarium oxysporum* f. sp. *albedinis* growth and survival. In vitro, shrimps shell, vetiver (*Chrysopogon zizanioïdes* L.) leaves and roots effect resulted in complete inhibition of spores germination and in 26, 27 and 56% decreased mycelial growth respectively for 15, 10 and 5 g/L. Amending soil with shrimps shell at 1% (wt/wt) suppressed completely *Fusarium oxysporum* f. sp. *albedinis* and resulted in 26.5% increased antagonistic actinomycetes population.

INTRODUCTION

In southern Morocco, date palm is grown in almost all oases. It is a source of income for more than one million people. Unfortunately, Bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis*, is a destroying factor of date palm. It is said that this vascular disease has killed more than ten million palms in Morocco and three million in Algeria since its onset in the 18th century. Moreover, because of the disease, some cultivars have become extinct and others are currently endangered. This would result in genetic erosion which will end up affecting the whole ecosystem and life in oases.

To control this soil borne disease, research carried out in Morocco many years ago led to select new resistant cultivars and clones (Sedra, 2003, 2005). However, this resistance could be broken by new strains of the pathogen, for the latter is in perpetual genetic evolvement. Also, some cultivars of quality and high commercial value remain the target of the devastating fungus.

A strategy for sustainable and effective control of this problem will allow growing high quality and sensitive cultivars. Soil treatment before planting and its regular amendment with fungal biocide organic amendments throughout date palm plantation life is likely to protect the latter from eventual Bayoud attacks.

Soil solarization and fumigation have been reported as effective means of fighting soil borne diseases (Duniway, 2002; Hamm et al., 2003; Shachaf et al., 2007). Their combination is reputed to be more effective (Stevens et al., 2003; Chellemi and Mirusso, 2006; Shachaf et al., 2007). Moreover, many organic amendments have shown their toxic effect toward plant disease soil dwelling fungi. These amendments could be either plants debris (Lodha et al., 1997; Coelho et al., 1999) or animal wastes such as crustacean shells (Huang et al., 2006).

The objective of this study was to evaluate the lethal effect of soil solarization and metam sodium on survival of total soil-dwelling fungi, especially *Fusarium* spp., in open field. Also, aiming at selecting some biocide soil amendments, many natural products were tested for their effect on *Fusarium oxysporum* f. sp. *albedinis* growth and survival, in laboratory conditions.

MATERIALS AND METHODS

Soil Solarization and Fumigation

1. Treatments and Experimental Design. The study was carried out in an experimental station in southern Morocco during the warmest months of the year (July and August). The field had previously been planted with date palms that had been completely destroyed by Bayoud disease. A randomized complete block with three replicates (blocks) was used, and the size of each treatment plot was 9 m^2 (3×3 m). Four treatments were applied in each block as well as the control. The first one is soil solarization (sol). It consisted of watering the plots to saturation and covering them with 40 microns thin polyethylene film. In the second treatment, soil was fumigated with sodium (61 g/m^2) (Fum). This was done by diluting the pesticide in water and then spilling it over the plots. Afterwards, the latter were watered to deeply carry the fumigant. The third and the fourth treatments were two combinations of soil solarization and fumigation. The difference between the two combinations was the metam sodium doses, 40 g/m^2 (Sol+Fum1) and 61 g/m^2 (Sol+Fum2). The control was only watered to saturation. The experiment lasted 8 weeks.

2. Treatments Effects on Fungal Soil Population. Total soil fungi flora (TFF) in general and total *Fusarium* spp. (TF) were estimated as colonies forming units (CFU) per gram of dry soil to assess the effectiveness of the treatments. From each plot, samples were taken in three diagonal points at three depth levels, 0-20, 20-40 and 40-60 cm. All samples from the same depth level were mixed to make a composite sample for each treatment. After grinding and sieving, final soil particles were 80 microns or less. Aliquots (10 g) of the soil mixtures were suspended in 100 ml of sterile distilled water in 250-ml flasks and shaken for 30 min at 150 rpm; 10^{-3}, 10^{-2} and 10^{-1} fold dilutions were made respectively for 0-20, 20-40 and 40-60 cm depth samples. 1 ml was spread onto petri dishes containing Gzapeck medium (pH=4) to reveal total soil fungi or KOMADA medium for *Fusarium* spp. 10 replicates dishes were used for each treatment, depth and dilution. Petri dishes were maintained at ambient temperature for 5 to 7 days and the fungal colonies were counted.

Biocides Selection and Soil Amendment

Two natural products were tested for their in vitro effect on *Fusarium oxysporum* f. sp. *albedinis,* shrimps shell and vetiver (*Chrysopogon zizanioïdes* L.) roots and leaves. To meet this goal, different amounts of these products were incorporated to Gzapeck medium, after being grinded and crushed. After autoclaving, filtering and adjusting mixture pH (7.2), spores germination and mycelia growth of *Fusarium oxysporum* f. sp. *albedinis* were assessed on the amended medium. Shrimps shell was tested at arbitrarily chosen concentrations of 5, 10 and 15 g per L, and both vetiver leaves and roots were tested at a concentration of 5 g per L. As for spore germination, 1 ml of a diluted pre-prepared solution of spores was spread onto petri dishes containing Czapeck medium amended with either tested products. As for mycelial growth, a 4 mm plug from the advancing margin of the parasite on Gzapeck medium was seeded onto plates. Control colonies were grown in non-amended Gzapeck. The germinating spores were counted 4 to 6 days later and the diameter of the colonies was measured when the fastest growing ones reached the edges of the plates.

Another experiment was undertaken to evaluate the effect shrimps shell on Bayoud parasite and two antagonistic actinomyctes growth, in soil. In a flask, 300 g of shrimps shell amended soil (1%, wt:wt) were 2 h autoclaved, twice. The parasite and two strains of antagonistic actinomyces, previously prepared in talc, were introduced into the autoclaved soil, both at concentrations of 1000 CFU per g. The control was not amended. 3 replicates were used. One month later, parasite and antagonistic soil concentrations were evaluated using soil suspension dilution technique. The fungus was re-isolated in Gzapeck medium (pH=4) and actynomycetes in PYA (peptone yeast extract agar) medium. Results were shown as number of CFU per g of dried soil.

Data Analysis

Variance analysis was used to appreciate the effect of experiments, all treatments on soil fungi populations, both products on *Fusarium oxysporum* f. sp. *albedinis* in vitro growth and shrimps shell on the growth of the parasite and its antagonists, in soil. Newman and Keuls test (p=0.05) was then applied to define homogenous groups.

RESULTS

Soil Solarization and Fumigation

Most treatments have dramatically reduced the populations of total fungi in general and the *Fusarium* spp. flora I in particular (Fig. 1). The efficacy of all treatments exceeded 80% at 0-40 cm depth, with 100% efficiency for the combination of solarization and higher dose of metam sodium (Sol+Fum2). However, the deeper microorganisms are in soil, the less likely they are to be affected. Indeed, statistical analysis showed that at layer 40-60 cm, soil solarization and fumigation effectiveness dropped to 68% or less. Whereas combinations of these two means kept efficient to more than 80% in most cases.

Biocides Selection and Soil Amendment

Adding shrimps shell, vetiver leaves and roots to Gzapeck medium resulted in complete inhibition of *Fusarium oxysporum* f. sp. *albedinis* spores. Impact on mycelial growth was less important and proportional to the doses for all products (Table 1).

Amending soil with crushed shrimps shell eliminates completely the parasite and supports antagonists multiplication (Table 2).

DISCUSSION

To detect soilborne fungi we had to less dilute soil as the depth that it is taken from increases. This proves that most of these microorganismes live in upper soil layers. Many resarchers reported similar findings. They said *Fusarium* spp. are mainly concentrated at 0-30 cm depth (Hamm et al., 2003; Sign et al., 1996) wich is likely to increase the efficacy of soil treatments whose effects diminish at deeper layers.

Soil solarization using polyethylene film resulted in decreased total fungi in general and *Fusarium* spp. in particular. This result confirms others previously cited by many authors. Porras et al. (2007) showed that *Phytophtora cactorum* could be completely eliminated thanks to soil solarization. Israel et al. (2005) also asserted that *Fusarium oxysporum* f. sp. *cumini* is likely to be controlled by solar heating. Moreover, Tamietti and Valentino (2006) affirmed that solarization could obliterate 58 to 96% of fungal flora at the first 25 cm soil depth. In their findings, soil solarization decreased *Fusarium* flora populations from 7×10^3 to 0-25 CFU/g. Still, the weakness of soil solarization is its lower impact in deeper soil-dwelling fungi especially the Bayoud parasite.

Metam sodium killed most soil mushrooms. This fumigant is a generator of methyl isothiocyanate reputed to be highly active against most soil-living microorganisms (Duniway, 2002). A large amount of related studies have as important results as ours. Hamm et al. (2003) studied the effect of metam sodium on three potato fungal diseases caused by *Fusarium* spp., *Pythium* spp. and *Verticillium dahliae*. They found that this fumigant eliminated these pathogens even at 60 cm depth. Many other researchers reported findings related to *Fusarium* spp. elimination by metam sodium (Sumner and Phatak, 1988; Sumner et al., 1997). Yet, a relatively recent study provided evidence that methyl isothiocyanate is very tributary of soil conditions (Shachaf et al., 2007). This might be a major barrier to the reproducibility of such success if required methyl-isothiocyanate-release conditions are not met.

Combination of soil solarization and fumigation, even with lower dose, improved the efficacy of treatments at soil deeper layers. Such a result corroborates with many others. For instance, Eshel et al. (2000) found that the combination of soil solarization and smaller amount of metam sodium destroyed 100% of *Sclerotium rolfsii* and *Fusarium*

oxysporum f. sp. *basilici* inoculums at 0-40 cm depth. According to them, this may be a result of a synergetic effect between the physical and the chemical actions. Indeed, they suggested that the pathogens are weakened by the sub-lethal doses of one means and completely killed by the other means. To illustrate that, they said that the elongation of spore germinating-tubes is affected by sub-lethal doses of a given factor. As a result, spores become vulnerable to different aggressors (high temperatures, pesticides, thermo-tolerant antagonists and so forth). These explanations had argued many similar findings (Arora et al., 1996; Ben-Yephet et al., 1988). In our experiment, this phenomenon may have happened at 40-60 cm depth.

Shrimps shell and vetiver leaves and roots extracts inhibited in vitro growth of *Fusarium oxysporum* f. sp. *albedinis*. This let us think that these natural products have fungicidal effects that are likely to be exploited to fight against Bayoud disease. Results showed that vetiver roots are more toxic that its leaves. This fact suggests that roots exudates, in an eventual association date palm-vetiver, would help protect palm root infections.

Amending soil with shrimps shell resulted in complete elimination of the Bayoud parasite and increased antagonists populations. This may be due to the chitin contained in the carapace of this crustaceous. Bell et al. (1998) showed that fungi propagules number was reduced 30 days after soil chitin amendment. This also significantly reduced *Fusarium oxysporum* and celery yellow disease incidence, caused by *Fusarium oxysporum* f. sp. *apii*. In addition, Rose et al. (2003) produced evidence that 4% soil chitin amendment affected negatively *Fusarium* oxysporum f. sp. *radicis-cucumerinum*, inciting cucumber roots and stem rots. Furthermore, Zhou and Everts (2004) reported that chitin amendment completely obliterated *Fusarium oxysporum* f. sp. *niveum*, agent of watermelon wilt. When chitin is broken down it releases volatile gases such as ammoniac (NH_3) (Hampson and Coombes, 1995) which is reputed to be toxic for fungi (Hora and Baker, 1972). This may explain the results of our experiment.

In chitin amended soil, chitinolytic micro flora develops and multiplies (Frommel et al., 1991). Most of these microorganisms are bacteria and actinomycets which are known to be involved in chitin breakdown (William and Robson, 1981). This finding confirms our results. Indeed, actinomycetes antagonists surely used shrimps shell as a nutrient source to increase their populations. In turn they may have participated in suppressing the Bayoud parasite.

To grow best cultivars of date palm in Morocco soil desinfestation is an imperative action before planting, because most of these cultivars are highly sensitive. Combination of soil solarization and fumigation will help attain this objective. Soil amendment with selective biocide products such as crustaceous shells would be a good strategy to ensure a sustainable soil suppressiveness. This would happen because the selective product would support antagonists and defeat the pathogen. Moreover, crops association (example vetiver-date palm) would be a complementary way to further strengthen an integrated Bayoud control. However, additional research is to be done to search for more available biocides and to study ways and strategies that are necessary to practice soil desinfestation in large scale.

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Tables

Table 1. Effect of treatments on in vitro growth of *Fusarium oxysporum* f. sp. *albedinis*.

Mycelial growth (mean diameter of colonies, cm)					
Cont	Shr C1	Shr C2	Shr C3	Vet L	Vet R
9a[1]	7.9 b	7.7 c	6.7 d	6.6 d	4 e
% inhibition	12	14	26	27	56
Mean number of germinated spores					
Cont	Shr C1	Shr C2	Shr C3	Vet L	Vet R
12 a	0 b	0 b	0 b	0 b	0 b
% inhibition	100	100	100	100	100

Cont=control; Shr=Shrimps shell; Vet=vetiver; C1, C2 C3=Concentrations 1, 2, 3; L=leaves; R=roots.
[1] Means followed with the same letter were significantly different (p=0.05) by the Newman-Keuls test.

Table 2. Effect of shrimps shell soil amendment on *Fusarium oxysporium* f. sp. *albedinis* and its antagonists.

Parasite soil concentration ($\times 10^3$ CFU/g)		Antagonist-1 soil concentration ($\times 10^6$ CFU/g)	
Control	Treatment	Control	Treatment
7a[1]	0b	27a	34b
% decreased growth	100	% increased growth	26

[1] Means followed with the same letter were significatly different (p=0.05) by the Newman-Keuls test.

Figures

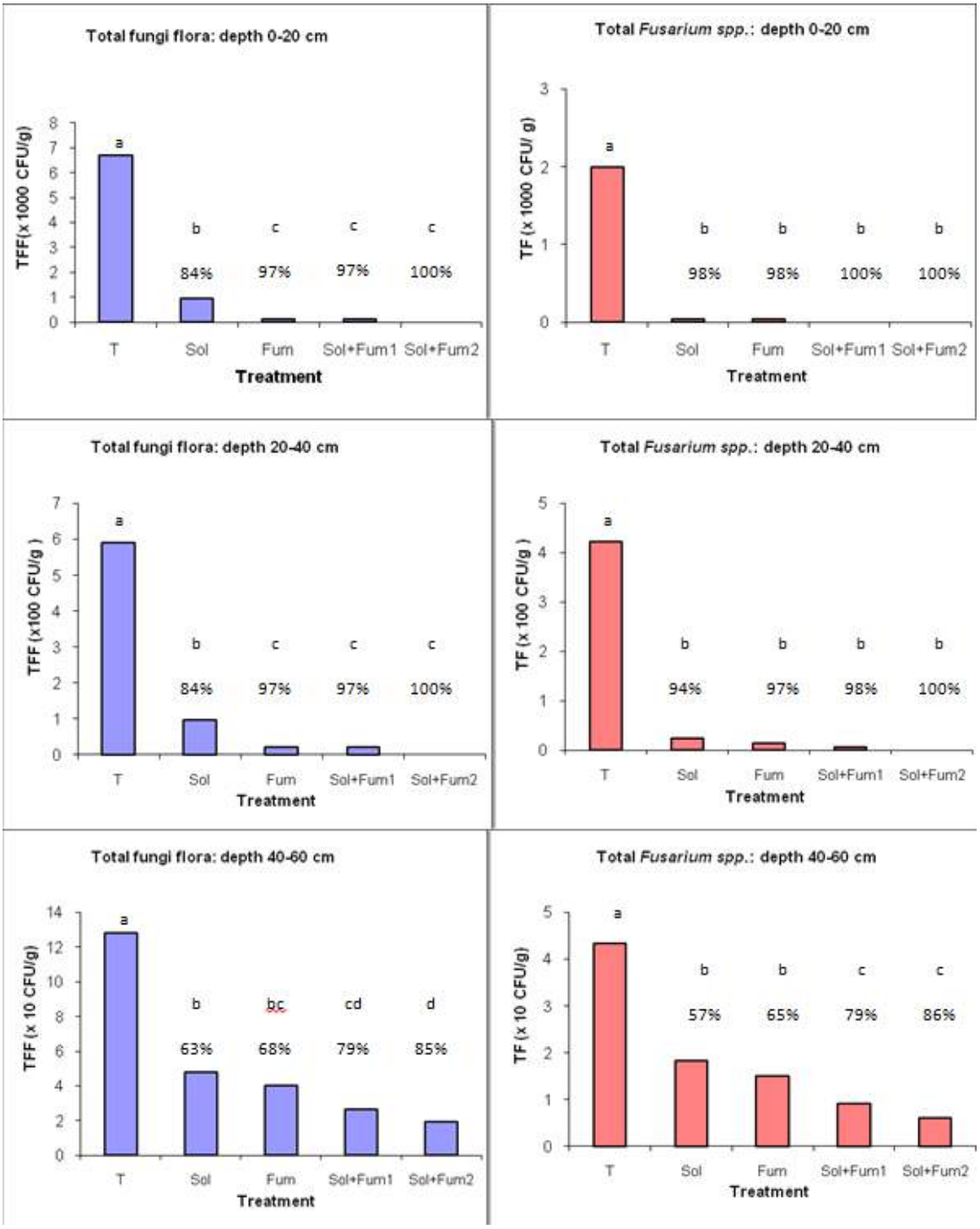

Fig. 1. Effects of soil treatments on survival of soil fungal flora. For each treatment at every depth, column topped with different letters represent treatments that are significantly different (p=0.05) by the Newman-Keuls test.

Effect of Different Kinds of Bunch Covering on Date Palm Fruit (*Phoenix dactylifera* L.) Moths Infestation Rate

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Keywords: date palm, bunch covers, moths, *Ectomyelois ceratoniae*

Abstract

One of the most depreciatory damage on date palm fruit quality in Morocco is the dates infestation by moths (*Ectomyelois ceratoniae*). The percentage of infested dates is estimated between 1 to 4% in the field and can reach more than 70% during fruit storage. The objective of the present study is to prevent the responsible adult insect to reach the fruits through bunch covering on the trees. For this purpose, four palm trees, 15 years old from the 'Najda', 'Bousthammi', 'Tademmamte', 'Bouslikhene', 'Admou' and 'Iklane' cultivars grown in the same environment of the Zagora Date Palm Experimental Station, were carefully chosen and used to study the effects of 4 different kinds of cover bags including: jute cover, and net covers with meshes as small as 3.8, 1.7 and 0.15 mm^2. The experiment was established at early khalal stage in a complete randomized block design. Samples of 400 dates were harvested in early October and damages were determined by counting the number of infested fruits per sample. The results of this three-years experiment showed that the lowest infestation rates (less than 1%) occurred with net covers of 1.7 and 0.15 mm^2 meshes while the highest damages (4.1 and 4.5%) occurred with jute covers and control respectively. Analysis of fruits dimensions and weights showed no significant differences between all treatments. These results were demonstrated and verified on 16 palm trees of 'Najda' and 'Jihel' cultivars grown at some growers' fields. These field experiments allowed dates production of less than 1% infestation rates. Accordingly, bunch covering with nets of 1.7 and 0.15 mm^2 meshes could be recommended to growers under similar conditions of the present study.

INTRODUCTION

During its life, date palm can be attacked by many harmful insects and pests which usually cause several damages to the trees and their fruits (Zaid et al., 2002). Among those insects and pests, moths have a special place because of the importance of damages they directly cause on the fruits. Sedra (2003) reported that moths, mainly represented by *Ectomyelois certaoniae*, cause damages of about 1 to 4% on dates still in the field. Adult moths are the responsible agents by putting their eggs on the fruits at maturity stage, during early July of each year (Zaid, 2002; El Abbassi, 1993). However, the biggest damages happen usually during fruit storage, where infestation rates can reach up to 70 or 80% in 9 months of storage (Sedra, 2003; El Abbasi, 1993). Furthermore, moths cause losses of more than 8% in dry weights of stored dates. If these dry weight losses appear low, the infestation rates remain however high (more than 80%). Such infestation rates seriously reduce the marketing performances of the whole date production (Fig. 1).

Thus, it has often been recommended to researchers to invest in this aspect in order to develop an appropriate inexpensive technique to effectively fight against dates moths infestation.

Several techniques were applied by researchers to fight against dates moths, such as chemical control, biological control, gamma radiations treatment, heat treatment,

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genetic control, male sterilization and the use of sexual pheromones. All these techniques require high skills as well as expensive sophisticated equipment, in addition to the adverse effect of some of them on the environment.

The present study was therefore undertaken, to develop an inexpensive technique, that is not harmful to the environment and can effectively contribute to the fight against dates moths infestation. It involves testing the effectiveness of some kinds of cover bags on date palm bunches in the fields.

MATERIALS AND METHODS

This experiment was undertaken at the Zagora Date Palm Experimental Station, located in the Draa Valley in the southeast of Morocco. Four palm trees, 15 years old from 'Najda', 'Bousthammi', 'Tademmamte', 'Bouslikhene', 'Admou' and 'Iklane' were carefully selected and used in this experiment. Bunches of the selected palm trees, were covered (Fig. 2) using four kinds of cover bags (Fig. 3), as follows: jute cover, net covers with meshes as small as 3.8, 1.7 and 0.15 mm^2.

On each palm tree, one or more bunches were not bagged to serve as controls. All the bags materials were ordered from the local market and their mesh sizes were measured in the laboratory using a precision magnifying glass. Subsequently cover bags of 1.5×1.5 m size were prepared.

The bagging operation was applied during early July, which is early khalal stage as recommended by Zaid and de Wet (2002).

All palm trees of the experiment were under the same agricultural practices applied for the palm trees of the Zagora Experimental Station.

The present assay containing the 4 treatments (kinds of cover bags), was installed in a randomized complete block design with 4 blocks (date palm trees) and 2 individuals (bunches) per experimental unit.

The experiment was also replicated at four growers' fields, particularly on 'Najda' and 'Jihel' date palm cultivars.

Dates were harvested in late October, and a 400 dates sample was randomly harvested from each bunch. After their opening, dates were examined under a precision magnifying glass, allowing noting the number of damaged dates per sample.

Data statistical analysis was performed using ANOVA test at 5%, with two classification criteria, while the means comparison was performed using Fisher's least significant difference at 5%.

RESULTS

Over the three years of the study dates infestation rates recorded from uncovered bunches showed that the 'Tademmamt', 'Najda', 'Bousthammi' and 'Bouslikhene' cultivars were more vulnerable to moths attacks than 'Admou' and 'Iklane' (Fig. 4).

The effect of bunch covering has been significant over the infestation rate of the harvested dates. Indeed, analysis of Figure 5 shows that the smaller the cover bag mesh the smaller the percentage of infested dates for all the tested cultivars.

Cover bags with meshes equal to 1.65 or 0.15 mm^2, reduced the dates infestation rate 3 to 4 times compared to the control. With such cover bags, the infestation rate was less than 1%.

Analysis of the characteristics of the produced dates shows that there was no significant effect of the bunch covering operation on dates' dimensions. Indeed, Table 1 shows that dates from covered bunches have similar average dimensions and weights to those of the control.

The installation of this experiment at farmers' fields in 4 oasis of the Draa Valley, as a verification trial, demonstrated the effect of bunch covering on reducing dates infestation by moths (Fig. 6). Cover bags with small mesh of about 0.15 mm^2, allowed farmers to harvest dates of less than 1% of infestation rate.

DISCUSSION

The bunch covering technique for fighting against dates moths, consisted on the idea of covering the date palm bunches by cover bags made from small mesh tissues to prevent the adult moths insects from putting their eggs on dates during the maturity stage. The use of this technique on several Moroccan date palm cultivars ('Tademmamt', 'Najda', 'Bousthammi', 'Bouslikhene', 'Admou' and 'Iklane') was effective in reducing the fruit infestation rates without causing any changes in the fruit characteristics (size, weight). Indeed, the obtained results in this experiment show that the use of cover bags with small mesh between 0.15 and 1.7 mm^2 substantially reduces the fruits attack, and the average dates infestation rates did not exceed 1%. In addition, dates from covered bunches retained the same characteristics (length, width and weight) as those of the control. Bunch covering has not only greatly reduced the date infestation rate, but also allowed keeping the quality of the produced dates.

These results are similar to those obtained by El Abbassi (1993) and Sedra and Zirari (1998) on 'Mejhoul' and 'Boufeggous' cultivars. They are also comparable to the recommendations made by other authors such as Zaid and de Wet (2002) and Sedra (2003).

The encouraging results of this study were verified at farmers' fields. The installed demonstration experiments clearly showed dates from covered bunches are 4 times less attacked by moths, since their infestation rate was less than 1%. Such dates were cleaner and thus more profitable on the market.

Based on these results, it seems reasonable to suggest that for a "green" fight against dates infestation by moths, bunch covering of date palm cultivars (such as 'Tademmamt', 'Najda', 'Bousthammi', 'Bouslikhene', 'Admou' and 'Iklane') using cover bags with reduced mesh sizes (between 0.15 and 1.7 mm^2) is effective and can be recommended on a large scale.

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Tables

Table 1. Average fruit length, width and weight according to the kind of cover bag.

	Date length (cm)	Date width (cm)	Average fruit weight (g)
Control	3,56	2,45	10,52
Jute cover	3,65	2,52	10,57
Net covers: 3.8 mm^2	3,71	2,57	10,61
Net covers: 1.7 mm^2	3,45	2,34	10,41
Net covers: 0.15 mm^2	3,53	2,42	10,50

Figures

Fig. 1. Moths damages date palm fruit.

Fig. 2. Bunch covering technique in the field.

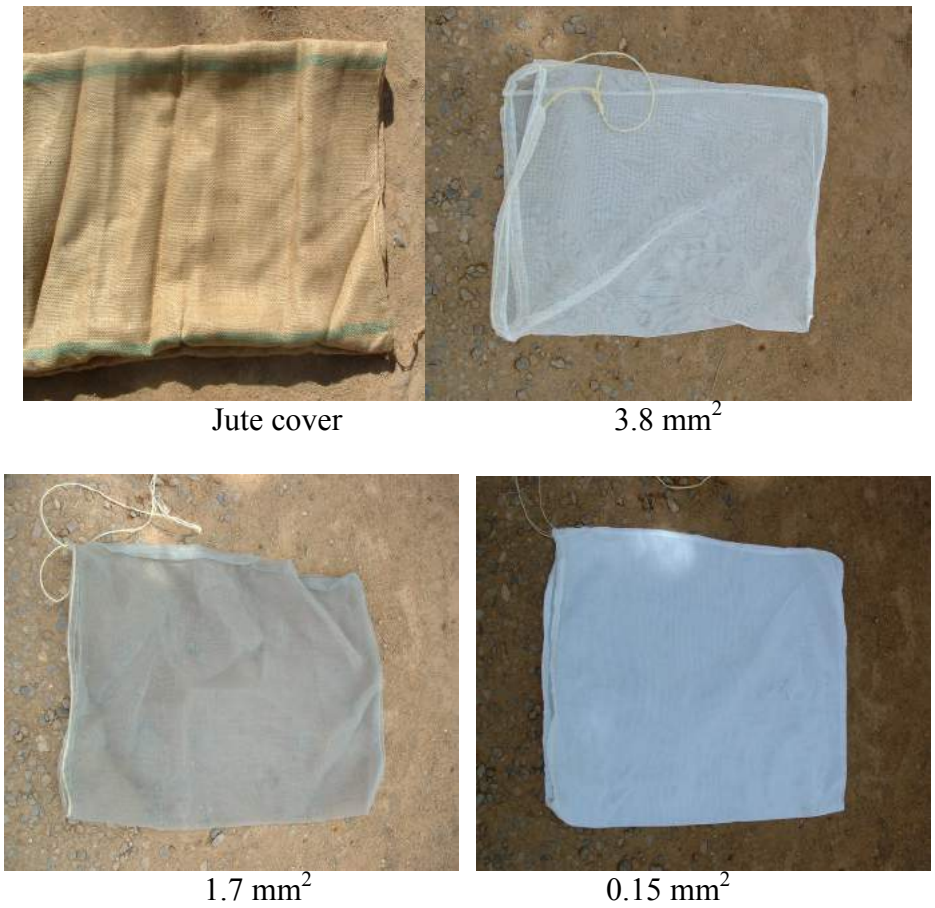

Fig. 3. The four kinds of cover bags used in the experiment.

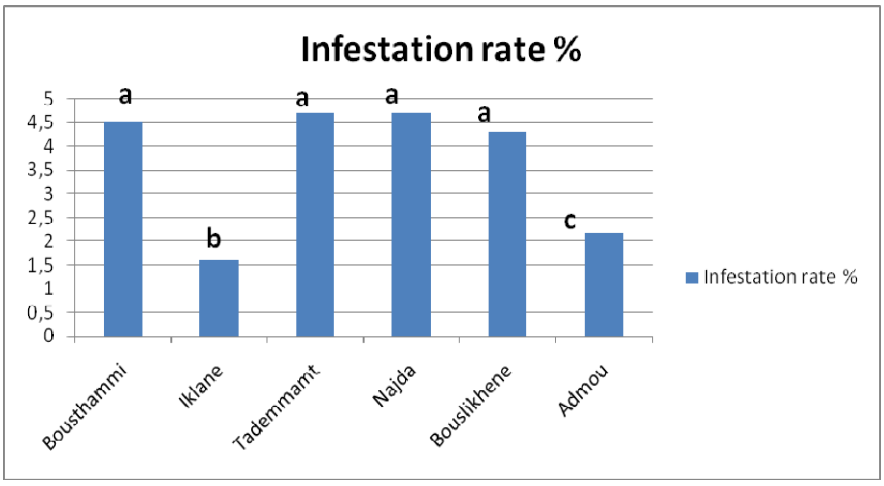

Fig. 4. Percentage of infested dates according to date palm cultivars. Values followed by different letters are significantly different (LSD at 5%).

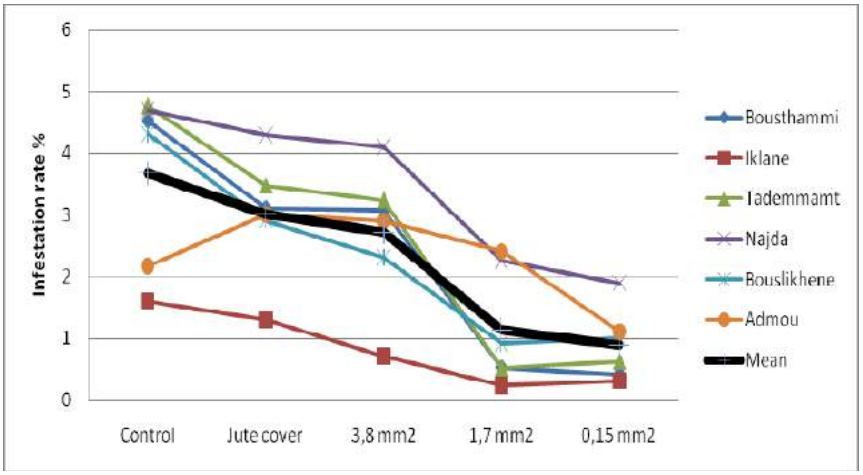

Fig. 5. Dates moths' infestation rate of the tested cultivars as affected by the bunch cover type.

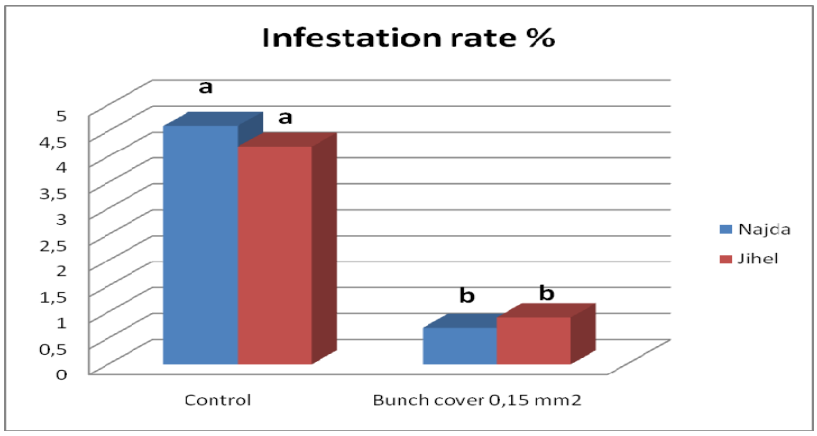

Fig. 6. Dates infestation rates as obtained at farmers' fields on 'Najda' and 'Jihel' date palm cultivars. Values followed by different letters are significantly different (LSD at 5%).

Assessment of Different Media for the Production of the Entomopathogenic Fungus *Beauveria bassiana* to Control the Red Palm Weevil *Rhynchophorus ferrugineus* (Oliv.)

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Keywords: *Beauveria bassiana*, culture, media, conidia, *Rhynochophorus ferrungineus*, red palm weevil, bioassay

Abstract

The growth of the entomopathogenic fungus *Beauveria bassiana* (B-SA3) in different media and subsequent quantity and viability of produced conidia was determined as well as virulence against the red palm weevil *R. ferrungineus*. Five ingredients in agar were tested; wheat, corn, barley, oat and soy bean. The standard SDYA medium was used for comparison.

The yield of cultured conidial was highest on media prepared by wheat followed by oat and least on barley. Viability of harvested conidia from all of the tested media, as determined by percentage germination ranged between 94.03-97.6% and relatively comparable with those obtained using SDYA. *B. bassiana* (B-SA3) conidia produced on the tested media although highly infective to *R. ferrungineus* adults was significantly less than that harvested from SDYA as exhibited by the calculated LC_{50} and LT_{50}. Of the considered ingredients, conidia cultured on wheat medium surpassed those produced on the other media (LC_{50} was 2.11×10^7 conidia/ml) followed by soy bean (1.06×10^8 conidia/ml). Least infectivity to the red palm weevil was by conidia produced on oat or corn medium, as LC_{50} was 2.81×10^8 and 3.37×10^8 conidia/ml, respectively. Mycosis was apparent on cadavers by the 7th day following death of all treated weevils.

INTRODUCTION

Date palm trees are infested by many insect pests as well as bacterial and fungal diseases leading to a great loss in their harvest and sometimes to damage beyond repair to the trees. In the past two decades the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*) has become a major key insect pest to date palm trees. This insect pest has been widely accepted as being the most devastating insect pest of date, coconut and oil palm trees throughout Asia (Kalshoven, 1950; Wattanaapongsiri, 1966; Abraham et al., 2002). In the mid 1980s *R. ferrugineus* was introduced to date palm trees in the Arabian Gulf Region. It quickly spread to Saudi Arabia, Iran and Egypt (Abraham et al., 1998; Murphy and Briscoe, 1999; Soroker et al., 2004). In 1997, the Arab Organization for Agriculture Development, AOAD, based in Sudan, set a project with the aim of controlling this destructive insect pest.

Due to the insect feeding habits inside the palm tree trunks, its control has been quite difficult; furthermore, early infestation cannot be discovered until damage has already been inflicted. Efforts for the control of the red palm weevil were focused on the use of traditional chemical insecticides or by eliminating infested trees. The use of food baited pheromone traps in both surveillance and mass trapping form a vital component of an IPM strategy against the red palm weevil (Abraham et al., 2002). Control of this pest is now more concerned with the use of biological control agents, such as the use of entomopathogenic bacteria, viruses, fungi or nematodes. *Beauveria bassiana* is well known as an entomopathogenic fungus with worldwide distribution with broad spectrum insecticidal activity (Li, 1989; Donaldson and Williams, 1981; Martin et al., 2000; Saleh

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and Alheji, 2004). This fungus is the anamorphic stage of *Cordyceps bassiana*, a teleomorph in the ascomycetous family, *Clavicipitaceae* (Sung et al., 2007). *B. beauveria* has proven to be effective for the control of many Coleopteran species, (Miranpuri et al., 1992a,b; Miranpuri and Khachatourians, 1994; Magra et al., 2004).

Use of entomopathogenic fungi in a bio-control program will require production of large amounts of inoculums. The components of the Sabouraud's dextrose yeast agar (i.e., SDYA) medium as a culture for the entomopathogenic fungus *B. bassiana* although very successful is relatively costly. Therefore, for the mass production of *B. bassiana* it was significant to experiment with other less expensive ingredients in the cultivation medium. Subsequently, the produced *B. bassiana* conidia on these media were evaluated in regard amounts obtained, viability and virulence on the red palm weevil.

MATERIAL AND METHODS

Insect Culture of the Red Palm Weevil *Rhynchophorus ferrugineus* (Oliv.)

The culture of the red palm weevil *Rhynchophorus ferrugineus* (Oliv.) was conducted under laboratory conditions at 27±2°C and 70±5% RH. Adult weevils were collected from infested date palm trees at a plantation located at El Kassasine, Ismailia Governorate, Egypt, by means of insecticide free food baited aggregation pheromone/ kaironome traps (Hanounik et al., 2000). The traps were partially buried around the trunk of the date palm trees at distances of 100 m. The traps were routinely inspected and the live trapped weevils were collected and transferred to the laboratory and reared according to Aldossary et al. (2009).

The Entomopathogenic Fungus *Beauveria bassiana* (B-SA3)

The entomopathogenic fungus Saudi Arabian isolate (B-SA3) of *B. bassiana* was used in the present investigation. This fungus was isolated from dead red palm weevils *R. ferrugineus* collected from date palm plantations at Al-Qatif province, at the Kingdom of Saudi Arabia by Hegazy et al. (2007) and which was identified and confirmed by CABI Bioscience UK.

Mass Cultivation of *Beauveria bassiana* (B-SA3)

Five seeds or grains, namely oat, barley, wheat, corn and soy bean were chosen to prepare a media for the cultivation of *B. bassiana.* The seeds or grains were thoroughly washed in running water and then allowed to dry at room temperature; subsequently they were ground to a fine powder by means of an electric mill. For the preparation of the media two weights, 20 or 40 g per L were considered for each tested ingredient, the grounded powder was mixed with 15 g agar per L of distilled water and autoclaved at 121°C for 20 min. The standard Sabouraud's dextrose yeast agar (i.e., SDYA) culture medium was used as a standard to compare the performance of the considered media.

The fungus *B. bassiana* (B-SA3) was grown on SDYA as well as the five other media for 96 h at 25±2°C, subsequently 5 mm in diameter discs were taken from the active growth region of the fungal colony and transferred in the centre of a 10 cm petri dish containing 20 ml in each of the prepared culture media to be tested. Each considered culture medium was presented by 3 plates and each replicated 4 times. After incubation at 25±2°C for 15 days, the conidia were harvested in sterile vials by scraping the surface of agar plates and the following factors were determined:

a. Dry weight (g) of produced conidia/plate.
b. Viability of conidia, assessed by germination test according to Goettel and Inglis (1997).
c. Sporulation of the fungus was determined by conidial counting as number of conidia/cm^2 of fungal growth. After incubation at 25±2°C for 15 days, conidia were harvested by flooding the sporulated culture with sterile distilled water containing 0.05% Tween-80 and dislodging conidia with a glass rod. Final conidial suspension volume was 25 ml and that was vortexed for 2 min to break spore chains into

individual spores to assure uniform mixing. Counting of conidia was conducted by the use of a hemocytometer slide under an optical microscope.

d. A bioassay of the *B. bassiana* conidia cultured on each of the considered media ingredient (i.e., corn, wheat, oat, soy bean or barley) as well that cultured on the standard SDYA medium was evaluated on adult *R. ferrungineus* weevils. A series of concentrations were prepared, 5×10^5, 5×10^6, 5×10^7, 5×10^8 and 5×10^9 conidia/ml of *B. bassiana* according to the method described by Marannino et al. (2006), the suspensions were used for inoculation within 1 h. The dipping technique was used, where 20 weevils were dipped for 20 s in one of the prepared concentrations. Treated specimens were then maintained separately and provided with small pieces of date palm wood as a source of food; each treatment was replicated 3 times. A control present by an equal number of weevils was included. Accumulated mortality percentage of treated weevils was recorded and corrected according to Abbott's formula (Abbott, 1925) and LC_{50} and LT_{50} values determined (Finney, 1952). Following the death of treated weevils their cadavers were incubated individually at 25°C in sealed moist petri dishes. After 7 days the specimens were examined to determine if the death was a result of infection by the fungus as confirmed by mycosis and/or 'mummification'.

RESULTS AND DISCUSSION

Weight of Produced *B. bassiana* Conidia

As seen in Figure 1 the medium prepared with oat or wheat produced the highest weight of *B. bassiana* conidia. Wheat medium was slightly superior when added at 20 g than oat medium, as an average weight of 0.138 and 0.127 g of conidia were obtained, respectively, however these values were significantly lower to conidia cultured on SDYA. Meanwhile, when the medium for the culture of conidia was prepared with 40 g of either of these two grains, the weight of cultured *B. bassiana* conidia was 0.176 g in the medium made with oat which was only slightly but insignificantly higher than that of the medium prepared with wheat, i.e., 0.171 g. It is noteworthy that the weight of conidia produced on either of these two medium was also insignificantly different than those produced on the standard SDYA medium (i.e., 0.174 g).

The medium made by 20 g of corn, barley or soy beans produced a markedly lower weight of conidia than the standard SDYA medium, i.e., 0.097, 0.089 and 0.086 g, respectively, as compared to 0.174 in SDYA; the difference between them was highly significant**.** Meanwhile, when 40 g of these considered ingredients were added, the weight of cultured conidia was higher in the medium containing soy bean followed by that made with corn and least in barley, i.e., 0.132, 0.122 and 0.092 g, respectively.

Number of *B. bassiana* Conidia/cm^2

The number of *B. bassiana* (B-SA3) conidia/cm^2 obtained when cultured on the different tested media varied (Table 1). The standard SDYA medium was the most efficient in producing the largest number of *B. bassiana* conidia i.e., 8.7×10^8 conidia/cm^2.

The tested media that were prepared with 40 g of oat or wheat produced the highest number of *B. bassiana* (B-SA3) conidia which was 7.9×10^8 and 7.6×10^8 conidia/cm^2, respectively. However, when either of these media was prepared at a lower weight of 20 g, that made with wheat was slightly superior to that of oat as the number of conidia was 6.4×10^8 and 5.5×10^8 conidia/cm^2, respectively.

Media prepared with 20 g of either soy bean or corn were similar in regard the number of cultured conidia being 3.3×10^8 and 3.1×10^8 conidia/cm^2, respectively. However, when prepared with double their quantities i.e., 40 g, of either soy bean or corn the former medium produced 5.5×10^8 conidia/cm^2 which was much more than those produced on corn (4.3×10^8 conidia/cm^2).

The number of produced *B. bassiana* (B-SA3) conidia cultured on a medium prepared with either 20 or 40 g barley was the lowest as only 2.9×10^8 and

3.1×10^8 conidia/cm^2 , respectively, were counted.

Germination Test of *Beauveria bassiana* (B-SA3) Conidia Cultured on Media Prepared with Different Ingredients

In the standard SDYA medium percentage of conidia germination was 98.2%, as seen in Figure 2, the germination of conidia cultured on any of the tested media ranged between 94.03-97.6%. The highest percentage of germination was detected in *B. bassiana* conidia cultured on a medium prepared with 40 g of oat, wheat or corn, i.e., 97.6, 97.6 and 97.5%, respectively. These percentages were comparable and insignificantly different than that of *B. bassiana* conidia harvested from SDYA medium. When the media were prepared with these mentioned ingredients (i.e., oat, wheat or corn) but at half their weight (20 g) the percentage of conidia was slightly reduced to reach 96.5, 97.3 and 96.7%, respectively. These results show that wheat medium was the least affected when prepared in lesser amounts. Lowest germination was detected in conidia cultured on soy bean medium, prepared in either 20 or 40 g, being 94.03 and 94.4%, respectively, which was significantly less than germination of conidia harvested from SDYA medium.

Bioassay of *Beauveria bassiana* (B-SA3) Conidia Cultured on Different Media

The virulence and infectivity of *B. bassiana* (B-SA3) conidia depicted by accumulative percentage mortality of adult red palm weevils treated with different concentrations of *B. bassiana* (B-SA3) conidia harvested from different media are exhibited in Figure 3 and 4 and were shown to be concentration dependent. Accumulated mortality was highest in red palm weevil treated by *B. bassiana* (B-SA3) conidia harvested from the media prepared with wheat followed by barley then soy bean in any of the tested concentrations.

The previous observation was confirmed by the determined LC_{50} values which were 2.11×10^7, 6.9×10^7 and 1.06×10^8 conidia/ml in the media prepared by 40 g of wheat, barley or soy bean, respectively, (Table 2). Meanwhile, when these respective mentioned ingredients when included in lesser amounts of 20 g, the LC_{50} values were higher, being 5×10^7, $1.96\times10^8$and 2.37×10^8 conidia/ml, respectively. However, conidia collected from the standard SDYA were much more infective to red palm weevil adults as a lower LC_{50} value of 3.75×10^6 conidia/ ml was calculated (Table 2).

Least infectivity was detected in red palm weevils treated with *B. bassiana* (B-SA3) conidia harvested from a medium made with oat or corn. The determined LC_{50} values were 2.81×10^8 and 3.37×10^8 conidia/ml when the medium was prepared with 40 g of the mentioned ingredients. Meanwhile, LC_{50} was a high of 8.38×10^8 and 8.66×10^8 conidia/ml when the medium was prepared with 20 g of either corn or oat (Table 2).

The time required to kill 50% (LT_{50}) adults of the red palm weevil treated with 5×10^9 conidia/ml of *B. bassiana* (B-SA3) should that conidia cultured on a medium made with 40 or 20 g of wheat caused the shortest LT_{50} which was 79.07 and 90.39 h, respectively, (Table 3). Conidia from the barley medium were slightly superior to that of soy bean when prepared with 40 g, i.e., 82.41 and 88.51 h, respectively. Meanwhile, when the medium was prepared with 20 g of either of these latter mentioned two ingredients; their LT_{50} values were insignificantly different being 97.95 and 98.17 h. respectively. The LT_{50} value of *B. bassiana* (B-SA3) conidia cultured on oat or corn media was slightly longer as compared with conidia cultured on the other considered media. It is noteworthy, that LT_{50} value of conidia harvested from the standard SDYA medium was the most superior as it was the lowest recorded, i.e., 73.74 h (Table 3).

Treatment of adults of the red palm weevils with *B. bassiana* (B-SA3) conidia harvested from different media at a concentration of 5×10^8 conidia/ml showed that similar to the previous higher considered concentration, conidia cultured on the wheat medium caused the lowest LT_{50} value. These values were 93.97 and 97.95 h when the medium was prepared with 40 or 20 g wheat, respectively (Table 4). The LT_{50} of conidia collected from a medium made with 40 or 20 g soy bean was 105.68 and 108.39 h which was slightly lower than that of conidia produced on barley (i.e., 107.4 and 112.46 h), prepared

in the respective mentioned weights. Also, similar to the previous results, conidia harvested from a medium made with corn or oat were the least virulent against the red palm weevil (Table 4). Again the virulence of *B. bassiana* (B-SA3) conidia harvested from the standard SDYA was 76.21 (Table 4).

Red palm adult weevils treated with *B. bassiana* (B-SA3) conidia harvested from tested media and at a concentration of 5×10^7 conidia/ml followed the same trend in regard LT_{50} values as the other two higher tested concentrations but took a longer time. At this concentration the shortest LT_{50} time was detected with 40 g of the wheat medium (i.e., 119.67 h) and the longest time was 195 h for conidia produced on 20 g corn. These periods were relatively comparable to conidia produced on 40 or 20 g oat as LT_{50} was 185 and 190 h, respectively (Table 5). Conidia of *B. bassiana* (B-SA3), at the same considered concentration, harvested from the standard SDYA was more virulent to red palm weevil adults, LT_{50} was 85.7 h.

The LT_{50} value could not be calculated for the lower concentrations (i.e., 5×10^5 and 5×10^6 conidia/ml) due to low mortality of treated adult weevils.

Mycoses on Treated *R. ferugenesis* Cadavers

As seen in Figure 5 the highest percentage of visible aerial mycelium (mycosis), on the 7th day post mortem, was detected on cadavers of weevil's treated by *B. bassiana* (B-SA3) conidia cultured on the standard SDYA medium. These percentages were 100% at the two highest concentrations of 5×10^9 and 5×10^8 conidia/ml, but declined to 87.5% at the lower concentrations 5×10^7 conidia/ml.

Of the tested media the highest percentage of mycosis was 77.78% on cadavers of the red palm weevils, by the 7th day following their death, when treated with 5×10^9 *B. bassiana* (B-SA3) conidia/ml harvested from a medium prepared with 40 g of wheat. This was followed by a slightly lower percentage of 75% by *B. bassiana* (B-SA3) conidia cultured on barley medium. Also, at this considered concentration, 71.43% of cadavers of weevils treated with conidia produced on either 40 g of oat, soy bean or corn exhibited mycosis.

The percentage of mycosis on cadavers of the red palm weevil gradually decreased with a decrease in the administered conidia concentrations as well as when the media were prepared with a lesser weight of the component. The decrease was at a sharper rate in weevil cadavers treated by conidia produced on oat. However, at the lower concentration of 5×10^5 conidia/ml, none of the dead treated weevils displayed mycoses.

Use of fungi in practical biological control programs will require production of large amounts of inoculum (Batista-Filho et al., 1988; Jenkins et al., 1998). In the present work, media prepared with available seeds or grains, i.e., wheat, oat, corn, soy bean or barley in agar were considered for the culture of *B. bassiana* conidia. The SDYA medium was taken as a comparison. From the fore mentioned results, it could be deducted that medium made with 40 g of any of the considered ingredients were much more resourceful for the production of *B. bassiana* (B-SA3) than when prepared with the lesser weight of 20 g. Therefore, the following summation of results present media prepared with only 40 g. As depicted in Table 6, the medium prepared with 40 g wheat was the most efficient for the production of *B. bassiana* (B-SA3) to be used in a control program against the red palm weevil. This medium produced 17.2% conidia less in weight and 12.6% less in number than those produced on the standard SDYA medium. Furthermore, percentage conidia germination was comparable as it was only 0.6% less than the standard SDYA. In the conducted bioassay against the red palm weevil the LC_{50} value of conidia produced on wheat were 2.11×10^7 conidia/ml, and surpassed that of conidia produced on other components. Also, in this case, LT_{50} values were the shortest.

The culture medium prepared with 40 g oat could be taken in second position, as it produced the same weight of *B. bassiana* (B-SA3) spores as those obtained on SDYA but less with a slight 9% and a negligible 0.9% lower number of conidia/cm^2 and germination percentage, respectively, than the standard medium. However, the LT_{50} of conidia produced on this component (and also those cultured on corn medium) exhibited the

longest duration recorded as they ranging between 195-175 h.

A medium made with 40 g corn produced a very low number of *B. bassiana* (B-SA3) conidia as it was half the value produced on SDYA medium (i.e., 50.5%) but these conidia had a high germination percentage of 97.5%. The virulence of these conidia against red palm weevil adults was relatively low as detected by the determined LC_{50} value which was 3.37×10^8 conidia/ml.

Of the tested media, that prepared with barley medium produced the lowest number of conidia which was less by 64.4% than that produced on the SDYA medium. These conidia exhibited a high viability as depicted by germination percentage (96.1%) but their virulence against red palm weevil adults was low. In contrast LC_{50} value of conidia produced on soy bean was 1.06×10^8 conidia/ml when tested on the red palm weevil, which put their virulence in an enhanced position if compared with other tested media. The considered prepared media are not to be underestimated as their conidial yield and viability was relatively reasonable. The lesser infectivity of these conidia as compared to conidia harvested from the standard SDYA medium may be compensated by the addition of other additives; further studies are required to confirm this assumption.

Mycosis was apparent on 77.78-71.43% of dead treated weevils treated by LC_{50} values above 5×10^6 conidia/ml; it is most significant for the occurrence of mycosis on the cadavers of weevils so as to create an opportunity of spreading the conidia. This calculation would be most important when conducting a control program for the red palm weevil especially that male adults of this insect release an aggregation pheromone (Al-Jahr and Al-Rajeh, 2000), therefore, allowing close contact with other weevils and feasibility of fungal infection spreading between weevils. An experiment conducted by Andrei (2001) showed that *B. bassiana* conidia in the soil from cadavers of infected *Leptinotarsa decemilineata* (Say) adults caused high mortality to other insects.

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Tables

Table 1. Number of *B. bassiana* (B-SA3) conidia/cm^2 grown on media prepared with different ingredients.

Ingredient	N°. conidia/cm^2 cultured on media in two weights (g/L)	
	20	40
Oat	5.5×10^8	7.9×10^8
Wheat	6.4×10^8	7.6×10^8
Corn	3.1×10^8	4.3×10^8
Soy bean	3.3×10^8	5.5×10^8
Barley	2.9×10^8	3.1×10^8
SDYA	8.7×10^8	

Table 2. LC_{50} of red palm weevil adults treated with *B. bassiana* (B-SA3) conidia harvested from media made with different ingredients in two weights (40 or 20 g/L).

Component	Wt. (g/L)	LC_{50} conidia/ml	Slope	*r*
Barley	20	1.96×10^8	0.439	0.983
	40	6.91×10^7	0.414	0.993
Corn	20	8.58×10^8	0.391	0.995
	40	3.37×10^8	0.442	0.994
Wheat	20	5.00×10^7	0.441	0.995
	40	2.11×10^7	0.502	0.991
Oat	20	8.66×10^8	0.381	0.992
	40	2.81×10^8	0.414	0.979
Soy bean	20	2.37×10^8	0.460	0.988
	40	1.06×10^8	0.351	0.991
SDYA	-	3.75×10^6	0.640	0.990

Table 3. LT_{50} (h) values of red palm weevil adults treated by *B. bassiana* (B-SA3) conidia at conc. 5×10^9 conidia/ml, harvested from media prepared with 40 and 20 g/L of different ingredients.

Ingredient	Wt. (g/L)	LT_{50} (h)	Slope	*r*
Barley	20	97.95	6.182	0.998
	40	82.41	4.891	0.993
Corn	20	102.92	5.018	0.974
	40	90.36	4.794	0.980
Wheat	20	90.39	6.062	0.979
	40	79.07	6.880	0.998
Oat	20	94.08	2.268	0.997
	40	90.39	4.794	0.980
Soy bean	20	98.17	4.603	0.941
	40	88.51	3.430	0.963
SDYA	-	73.74	9.140	0.984

Table 4. LT_{50} (h) values of red palm weevil adults treated with *B. bassiana* (B-SA3) conidia at conc. 5×10^8 conidia/ml harvested from media made with different ingredients.

Ingredient	Wt. (g/L)	LT_{50} (h)	Slope	*r*
Barley	20	112.46	4.820	0.952
	40	107.40	4.923	0.999
Corn	20	123.30	3.734	0.977
	40	117.17	3.836	0.988
Wheat	20	97.95	6.182	0.998
	40	93.97	4.699	0.997
Oat	20	119.40	5.791	0.999
	40	117.17	3.836	0.988
Soy bean	20	108.39	6.980	0.992
	40	105.68	3.440	0.970
SDYA	-	76.21	5.600	0.918

Table 5. LT_{50} (h) values of red palm weevil adults treated by *B. bassiana* (B-SA3) conidia at conc. 5×10^7 conidia/ml harvested from media made with different ingredients.

Ingredient	Wt. (g/L)	LT_{50} (h)	Slope	*R*
Barley	20	170.00	5.915	0.978
	40	158.50	4.723	0.980
Corn	20	195.00	4.312	0.999
	40	175.00	3.340	0.999
Wheat	20	139.00	4.192	0.981
	40	119.67	3.578	0.991
Oat	20	190.00	3.340	0.999
	40	185.00	4.312	0.999
Soy bean	20	160.00	5.915	0.978
	40	138.99	4.190	0.981
SDYA	-	85.70	4.800	0.931

Table 6. Compiled data of the assessment of medium for the production of *B. bassiana* (B-SA3).

Ingredient	Wt (g/L)	Wt. spores (mg)	N° spores /cm^2	% germination	% decrease than standard medium SDYA			LC_{50}	LT_{50}		
					Wt.	N°. spores	% germination		5×10^9	5×10^8	5×10^7
Barley	20	0.089	2.9×10^8	95.07	48.80	66.6	3.3	1.96×10^8	97.95	112.46	170.00
	40	0.092	3.1×10^8	96.10	47.12	64.4	2.1	6.91×10^7	82.41	107.40	158.50
Corn	20	0.097	3.1×10^8	96.70	44.25	64.6	1.5	8.58×10^8	102.92	123.30	195.00
	40	0.122	4.3×10^8	97.50	29.80	50.5	0.7	3.37×10^8	90.36	117.17	175.00
Wheat	20	0.138	6.4×10^8	97.30	20.60	26.4	0.9	5.00×10^7	90.39	97.95	139.00
	40	0.171	7.6×10^8	97.60	17.20	12.6	0.6	2.11×10^7	79.07	93.97	119.67
Oat	20	0.127	5.5×10^8	96.50	27.01	36.7	1.7	8.66×10^8	94.08	119.40	190.00
	40	0.176	7.9×10^8	97.60	17.20	9.0	0.6	2.81×10^8	90.39	117.17	185.00
Soy bean	20	0.086	3.3×10^8	94.02	50.57	62.0	4.18	2.37×10^8	98.17	108.39	160.00
	40	0.132	5.5×10^8	94.40	24.10	36.7	3.8	1.06×10^8	85.70	98.17	124.45
SDYA	-	0.174	8.7×10^8	98.20	-	-	-	3.75×10^6	73.74	76.21	85.70

Figures

Fig. 1. Average weight of *B . bassiana* (B-SA3) conidia (g) cultured on media prepared with different ingredients.

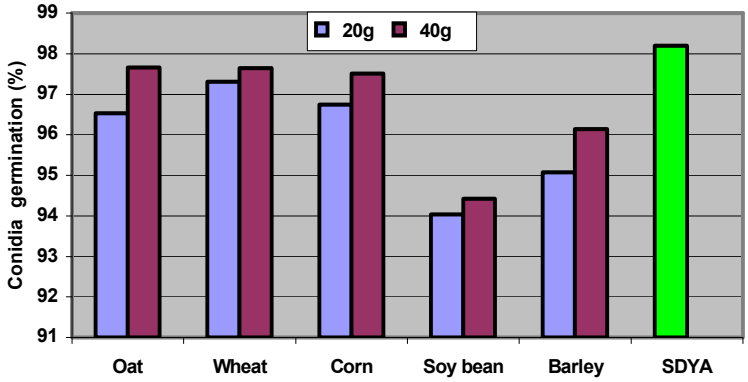

Fig. 2. Germination percentage of *B. bassiana* (B-SA3) conidia cultured on media prepared with different components.

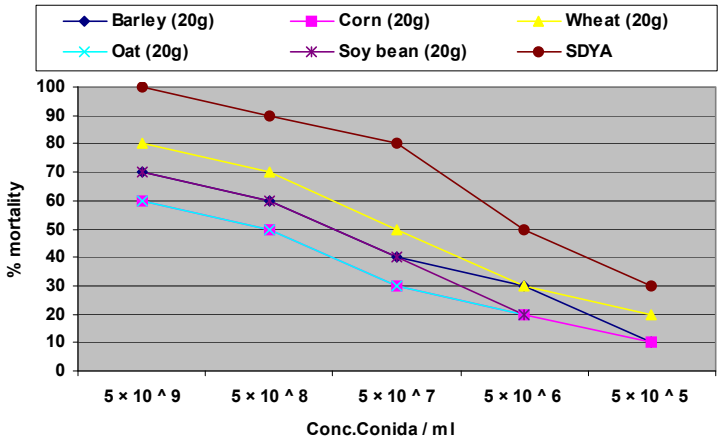

Fig. 3. Accumulated corrected percentage mortality of red palm weevils adults treated with different concentrations of *B. bassiana* (B-SA3) conidia cultured on media made with 20 g of different ingredients.

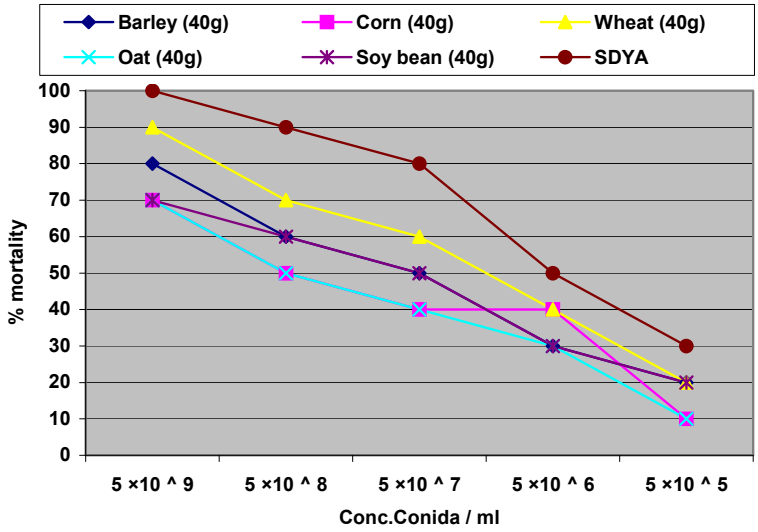

Fig. 4. Accumulated corrected percentage mortality of red palm weevils adults treated with different concentrations of *B. bassiana* (B-SA3) conidia cultured on media made with 40 g of different ingredients.

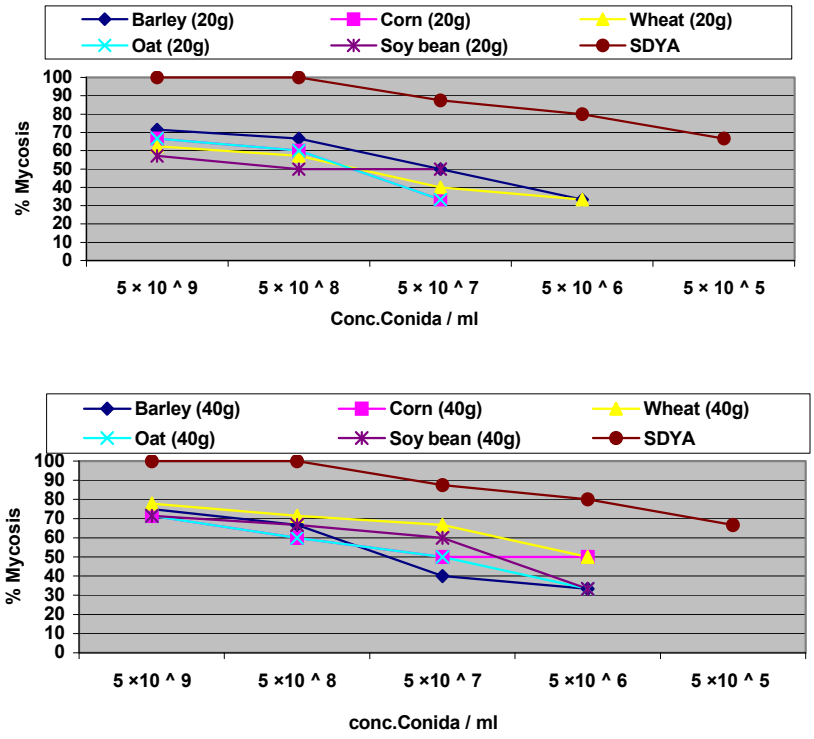

Fig. 5. Percentage of cadavers of red palm weevils exhibiting mycosis following treatment with different concentrations of *B. bassiana* (B-SA3) conidia cultured on different media.

Ecopalm Ring Machine: the Microwaves Technology for the Total Disinfestations of the Palm Trees Affected by the Red Palm Weevil

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Keywords: red palm weevil, *Rhynchophorus ferrugineus* Olivier, *Phoenix canarienis*, date palm infestation, date palm disinfestations, date palm treatments, plant treatment, ecological palm disinfestations

Abstract

The red palm weevil represents a serious threat for many countries where millions of dates and coconut palms are cultivated. Millions of palms around the world are ruthlessly attacked and damaged by the *Rhynchophorus ferrugineus* Olivier, causing great economic loss to the growers. All the efforts to control this pest have not yielded the desired results. Most of the methods currently applied and recommended by experts for the preventive and curative palms treatments are mainly based on pesticides and phytosanitary products use. The infestations control, using these products in urban environment and farms, is becoming more difficult due to the limitations imposed by legislation on use of plant protection products, which are polluting and harmful to health. Therefore, the necessity and the needs to seek more efficient and ecological alternative methods to address this serious emergency led our company, specialised for more than 20 years in high technology microwaves applications, to develop the patented Ecopalm Ring machine, based on the ecological microwave technology for the total disinfestations of the palms affected by the red palm weevil. Using the Ecopalm method has led to promising results in the fight against the red palm weevil by eradicating all the individuals hosted in the infested trunk, in whatever stage of their development they are found. The microwaves destroy selectively the eggs, larvae, pupae and adults, breaking and interrupting their life cycle completely, without causing any collateral damage to the palm treated, which is then saved. The Ecopalm method is very effective if used for preventive treatment of healthy palms, curative treatment of infected palms, and decontamination treatment of palms beyond recovery. An important aspect is the pure ecological method implemented, totally alternative to all methods known so far. No pesticides are used, no pollution is produced, and no harmful residues are left.

INTRODUCTION

The red palm weevil is a serious threat to many countries where millions of dates and coconut palm trees are cultivated. All the efforts to control this pest have not yielded the desired results. Most of the methods currently applied and recommended by experts for the preventive and curative palm treatments are based on pesticides and phytosanitary products.

The Ecopalm Ring machine (Fig. 1) is a circular ring-shaped device and consists of two articulated modular sectors, to enable the ring to surround the palm trunk and to make the treatment in depth. The height of the device is 80 cm , the treatment covers a trunk portion of 1.05 m, the internal diameter of the ring is 90 cm. The two articulated segments are equipped with electrical microwaves generators, called magnetrons, which generate high frequency electromagnetic waves necessary to eradicate the infestation.

The use of the Ecopalm Ring machine for the disinfestations of the palm trees

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from the red palm weevil is an efficient solution and an ecological alternative to all the methods actually used to control this dangerous infestation.

The objective of the work is to illustrate the promising results we have achieved in using Ecopalm technology, and to demonstrate the effectiveness of this method if used for preventive treatment of healthy palms, for curative treatment to recover infested palms, and for decontamination treatment of palms in advanced stage of infestation, beyond recovery.

MATERIAL AND METHODS

The machine is quickly placed around the infested part of the trunk, and started. The energy generated in high frequency electromagnetic waves, commonly named microwaves, travels quickly toward the centre of the palm trunk and penetrates in depth. The microwaves travel like in a fast track, across the air present in the cavities in a faster way than in the fibers of the plant, and go to strike directly all the individuals hosted there. The microwaves interact selectively with the water molecules that make up the organic materials of the pest. The overheating and hyperthermia generated by the microwaves' vibrations ensure the mortality of all red palm weevil individuals present in the trunk, whatever the stage of their development is, like eggs, larva, pupae and adult, interrupting completely their life cycle and giving the infested palm trees the possibility to recover completely, if they are not found in an advanced stage of the infestation.

Four different testing treatments of four different palms from the species *Phoenix canariensis*, have been effectuated. All trees were infested with the *Rhyncophorus ferrugineus* Olivier (red palm weevil), and were found in different stages of the infestation.

The treatments consisted in using the Ecopalm Ring machine by surrounding the infested part of the trunk, and then in starting the application. The temperature, on the external layer of the trunks was maintained at 60°C for 30 min, which is the necessary time to devitalize the pest in this case.

The first two testings were done as curative treatments, and have been effectuated to demonstrate the effectiveness of the Ecopalm method to eradicate totally the red palm weevil infestation from the palm, to destroy the pest in whatever stage of its development was found, and above all, to demonstrate how the palm totally recovered. During these two applications, the temperature in the two trunks was maintained at 60°C for 30 min, which is the time normally required by this method to devitalize the pest for this case.

The third testing was effectuated to demonstrate the effectiveness of the Ecopalm method to decontaminate the palms trees in an advanced stage of infestation and beyond recovery.

The last testing was done in over-treatment to verify the endurance level of the palm, and to demonstrate the harmlessness of the microwaves for the plant tissue, even when the treatment time was quadrupled from 30 min necessary to eradicate the infestation to 120 min, and when the temperature on the external layer of the trunk was raised from the necessary 60 to 85°C.

All the palms used were subjected before the treatment, to husking, and cleaning. An infrared probe was placed near the infested part of the trunk, on the external layer to detect the temperature trend, during the treatment.

Curative Treatment for Palm A

Palm A was infested and attacked strongly in the central bud. It was treated maintaining the temperature of the external layer of the trunk at 60°C for 30 min with the Ecopalm Ring machine. The palm was left alive and subject to periodic controls, every 20 days for a total of 90 days to assess the effects of the post-treatment intervention and to observe carefully the vegetative shooting, the development and the growth of its leaf apparatus. Three months later, in the spring time, the palm arrogantly pushed out new preponderant buds, one year after the treatment, the palm was totally recovered (Fig. 2).

Curative Treatment for Palm B

Palm B was infested and attacked strongly in the central bud. The palm was treated maintaining the temperature of the external layer of the trunk at 60°C for 30 min with the Ecopalm Ring machine. Once the treatment was completed, the trunk was covered with several layers of polyethylene film, to maintain the new thermal state. After 48 h from the end of the treatment, the trunk was cut into sections to verify the result of the disinfestations.

During the dissection of the trunk, 85 individuals from *Rhyncophorus ferrugineus* species (red palm weevil), were found in various development stages of their life cycle. All the individuals hosted in the trunk were found dead from which, 19 adults, 46 larvae, and 20 pupae (Fig. 3) The cavities created by the larvae, and by the exudates mounds have been affected by the microwaves in a greater extent than the surrounding vegetation which remained fresh, flourishing and hydrated. The microwaves travel like in a fast track, across the air present in the cavities in a faster way than in the fibers of the plant, and go to strike all the individuals hosted there. The internal layers of the treated trunk were heated in a smaller extent than the stages of the red palm weevil individuals, due to the different dielectric characteristics they have, and the lower amount of water molecules present in the vegetables than in the insect, consequently the palm tree did not suffer any damage.

Decontamination Treatment for Palm C

Palm C was in an advanced stage of infestation with the red palm weevil and beyond recovery. The palm was treated maintaining the temperature of the external layer of the trunk for 30 min at 60°C with the Ecopalm Ring machine. Once the treatment was completed, the trunk was covered with several layers of polyethylene film, to maintain the new thermal state. After 48 h from the end of the treatment the trunk was cut into sections to verify the result of the disinfestations.

During the dissection of the trunk, 203 individuals from *Rhyncophorus ferrugineus* species (red palm weevil), were found in various development stages of their life cycle. The number of the individuals from the red palm weevil found dead was 198 from which 82 adults, 11 larvae, 10 pupae, and 95 individuals between pupae and adults in the cocoons (Fig. 4). 5 adults were found alive. It is to specify that the 5 adults alive have been found in a cavity, below the lower edge of the machine, and during the examination they were in an evident state of sufferance. These five individuals were kept under close observation for 24 hours. 2 individuals died within the first 12 h, the remaining 3 within the following 24 h. This final result led the mortality rate to 100%.

Overtreatment for Endurance Level Verification for Palm D

This testing was done in overtreatment to verify the endurance level of the palm D, and to demonstrate the harmlessness of the microwaves for the palm plant tissue, even when the treatment time was quadrupled from 30 min necessary to eradicate the infestation in this case to 120 min, and the trunk temperature was raised from the necessary 60 to 85°C. The palm was left alive to assess the effects of post-treatment intervention and to observe carefully the vegetative shooting of the plant, the development and the growth of the leaf apparatus.

The palm was subject to periodical controls for 9 months. The leaves which have been over treated for 120 min continued to grow regularly and to have a normal vegetation in the upper part of the treated area with the microwaves. A certain number of these leaves branches have been dissected, and it was noted immediately that beneath the external layer treated and slightly dehydrated, due to the oversized microwaves irradiation, the vegetal fibers remained flourishing and green, with a regular lymphatic flow (Fig. 5).

CONCLUSION

The results achieved with the Ecopalm microwave technology are promising, it is

a new technology, for the first time applied on plants alive for disinfestations. It is an efficient ecological solution and an alternative to all the methods actually used to control the red palm weevil infestation. All the efforts to control this pest have not yielded the desired results yet. This method is ecological and clean, no harmful and toxic residues are left during and after use. It is safe for operators and for citizens. It can therefore be used in public and private places.

The Ecopalm method is efficient and will lead to promising results for the curative treatment of infested palms. The treatment eradicates the red palm weevil when the infestation is in progress and gives the possibility to save the palm. The method is easy and fast, in a few minutes the infestation is granted, and ensures the mortality of all the red palm weevils present in the trunk, in whatever stage of their development they are found, like eggs, larvae, pupae and adult, breaking and interrupting completely their life cycle. The palm does not suffer; the parts of the trunk and the branches treated with the microwaves remain fresh, flourishing and hydrated. The palm treated in time recovered, pushed out arrogantly a new preponderant bud, and returned to be a perfectly normal and healthy tree, in less than one year.

The Ecopalm method is also efficient for the preventive treatment of healthy palms. After pruning the fronds, the branches that remain on the palm tree have a high rate of humidity, which encourages the diffusion of fungi and bacteria. These bacteria emit olfactory molecules that attract the red palm weevil. Palm trees are therefore particularly exposed to the attack. In natural conditions, it takes a palm tree several months to heal from pruning and to dry out the humidity caused by mould and bacteria. The use of the Ecopalm method, after cutting off the fronds, helps to eliminate this problem, and to sterilize the trunk surface from mould, bacteria and rot, in just five minutes.

The Ecopalm method is efficient for the decontamination treatment of palms before elimination found in advanced stage of infestation and beyond recovery. The use of the Ecopalm method in this case, could be an alternative to the methods actually used in many countries consisting in cutting the trunk into many pieces and incinerating it. This operation is long and harmful, and is not efficient against the dispersal of the adult weevils.

The use of Ecopalm technology, with the implementation of the three methods of treatments above recommended, and following an accurate and well studied plan, helps in a first stage to slow down the advancement of the infestation, and contributes, in a second stage, to defeat it completely giving the great opportunity to rehabilitate entire infested areas.

ACKNOWLEDGEMENTS

Thanks to Prof. Emilio Caprio and to Prof. Rita Massa, respectively from the Department of Entomology and Agriculture Zoology and the Department of Physical Sciences, University of Napoli Federico II, Italy.

Figures

Fig. 1. The Ecopalm Ring machine is a circular ring-shaped device.

Palm A: Three months after the treatment. Palm A: one year after the treatment.

Fig. 2. Curative treatment of Palm A. Three months later, in the spring time, the palm arrogantly pushed out new preponderant buds, one year after the treatment, the palm was totally recovered.

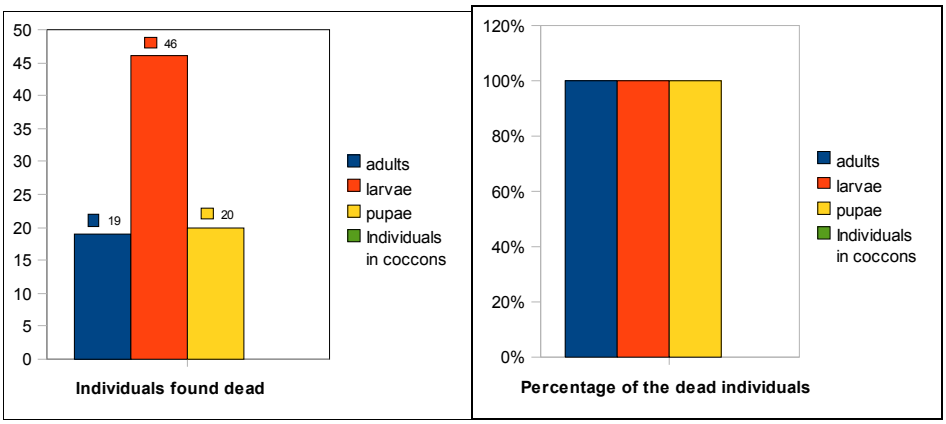

Fig. 3. Curative treatment of Palm B. Results of the inspection 48 h after the treatment. The total individuals from the four stages found dead, were 85 from a total of 85.

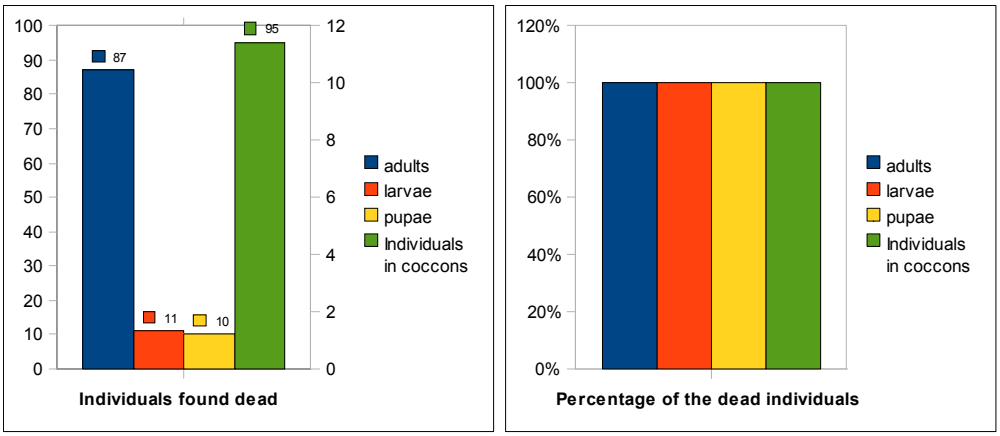

Fig. 4. Decontamination treatment of Palm C. Results of the inspection 72 h after the treatment. The total individuals from the four stages found dead were 203 from a total of 203.

Regular lymphatic flow, leaves grow regularly.

Vegetation around cavities remains green.

Fig. 5. Overtreament for endurance level of Palm D. Beneath the external layer treated and slightly dehydrated due to the oversized microwaves irradiation, the vegetal fibers remained flourishing and green, with a regular lymphatic flow.

Wings of Red Palm Weevil (RPW) and Their Behavior as Semiconductor

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Keywords: red palm weevil (RPW), elytron, EDAX, semiconductors

Abstract

Studies on wings of red palm weevil (RPW), *Rhynchophorus ferrugineus*, showed that elytron and hind wings have tapered, papillae structures that are distributed in array. By using EDAX analysis we found that the elytron of the insect composed of different elements mostly transient elements composed of a combination of C, Cl, Fe, Cu and Re materials with percentages ~75.8, 2.8, 3.3, 10.2 and 7.8% respectively. The EDAX analysis of the insect hind wing showed that the hind wing is composed of a combination of C, Cu, K, Zn and N materials with percentages ~12.7, 46.5, 1.2, 17.4 and 22.2% respectively. While the EDAX analysis without using gold as adhesive showed that the elytron has (C, O, Cl, K, Cu) with percentages of 70.92, 27.67, 0.32, 0.38 and 0.71 respectively, and the hind wing contains C, O, Si, P, Ca, Cu with the percentage of 64.49, 32.02, 0.12, 0.22, 2.80 and 0.35 respectively.

Potassium (K) was found in the elytron in the form of K MAD and this element has a photoelectric effect and means that the elytron needs any light to have energy that affects K. While the hind wing needs light and temperature hence its composition contains P in the form of GaP and Si in the form of SiO_2. Si would be the main component of the solar system and P would be behave as semiconductor. So, wings of RPW showed to have a photo-energy effects and semiconductor behaviour.

INTRODUCTION

A number of striking optical effects are produced by nanometer-scale architectures found in biological systems. These include antireflective arrays in some insects (Bernhard et al., 1965; Yoshida et al., 1997), light collecting lenses in a species of brittle-star (Aizenburg et al., 2001) and partial photonic band gap structures that give rise to strong iridescence (Kinoshita et al., 2002) or even polarized reflection of specific colures in some butterflies (Sweeney et al., 2003). There is currently a resurgence of interest in naturally occurring photonic structures, given the inspiration that they may provide for novel technological applications (Vukusic et al., 2003). Many of these systems are made out of chitin, which is the second most abundant naturally occurring polymer after cellulose (Kumar, 2000). In particular, the cuticle of insects is a self assembled nanocomposite of polysaccharides (i.e., chitin) and proteinaceous materials (Mayer et al., 2002). Chitin and its derivatives, such as chistosan, are attracting growing interest as versatile new functional materials with a number of potential applications in various industrial and biomedical fields (Vincent et al., 2004).

Scientists have discovered that their research to build photonic crystals was redundant. Mother Nature had already produced them with the ideal diamond-like structure, to decorate a beetle's carapace (Alexander, 2008).

Taxonomic and phylogenetic distribution with discussion of the putative functions at evolutionary pathways by which iridescence has repeatedly arisen in beetles is provided by Seago et al. (2009).

The aim of the work is to study the structure of wings (elytra and hind wings) of

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the RPW, its electrical properties and behavior as semiconductor.

MATERIALS

Culture Maintenance of RPW

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, is a species of beetle. It is relatively large, between two and five centimeters long, and of a rusty red colour.

A rearing colony was established according to Gindin et al. (2006), the colony of RPW has been reared at the Pests and Plant Protection Dep., National Research Centre, Cairo, Egypt.

Electron Microscopy

In case of scanning electron microscope (SEM) imaging, a square of dried tissue from the elytra and hind wings of RPW (~3×5 mm^2) was excised and mounted on an aluminum pin-type stub with double-sided adhesive, then sputter coated with 7-10 nm of platinum, before being imaged using a JEOL (Peabody, MA) 6300 field emission SEM at 8 kV.

The surfaces of RPW wings (elytra, hind wings) samples were examined using Scanning Electron Microscope (SEM).

Electric Properties

The Electrical conductivity (AC) of the elytron, hind wing and whole body larva were studied using a computerized LRC meter (Hioki model 3532-50 LCR Hi Tester) at different frequency from 42 Hz to 5 MHz at room temperature.

RESULTS AND DISCUSSION

Morphological Analysis

1. Scanning Electron Microscope (SEM). The samples were coated with gold as an adhesive and electronic conductor.

Figure 1a,b shows SEM images of the surfaces of RPW wings containing tapered, papillae structures that are distributed in a quasi-periodic array, for the dorsal elytron of the insect, which appeared as regular cells. While, the ventral side of the same wing showed that a smooth surface has some small hairs (Fig. 1.c,d).

By using EDAX analysis we found that the elytron of the insect is composed of different elements mostly transient elements composed of a combination of C, Cl, Fe, Cu and Re materials with percentages ~75.8, 2.8, 3.3, 10.2 and 7.8%) respectively (Fig. 1e).

Figure 2a,b showed SEM images for the dorsal hind wing of the insect, which appeared as smooth surface, while the ventral side of the same wing showed tapered, papillae structures that are distributed in array (Fig. 2c,d).

The EDAX analysis of the insect hind wing showed that the hind wing also consists of different elements mostly transition elements but it has a larger amount than elytron, they are composed of a combination of C, Cu, K, Zn and N materials with percentages ~12.7, 46.5, 1.2, 17.4 and 22.2%) respectively (Fig. 2e) and these materials would be a good conductor to the voltage and heat. By this combination of materials, the hind wing of the adult insect has the ability to absorb the light energy and turn it to electrical energy.

Note: The EDAX analysis, showed that the elytron has C, O, Cl, K, Cu with the percentage of 70.92, 27.67, 0.32, 0.38, 0.71 respectively (Fig. 3), without using gold as adhesive. At the same time EDAX of the hind wing under the same conditions, showed that it contains C, O, Si, P, Ca, Cu with the percentage of 64.49, 32.02, 0.12, 0.22, 2.80, 0.35 respectively (Fig. 4).

Potassium (K) was found in the elytron in the form of K MAD and this element has photoelectric effect and means that the elytron needs any light to have energy that affects K.

While the hind wing needs light and temperature hence its composition contains P in the form of GaP and Si in the form of SiO_2. Si would be the main component of solar system and P would be behave as semiconductor.

Electric Properties

1. Electrical Conductivity. The AC conductivity of the elytron, and hind wing were studied using a computerized LRC meter (Hioki model 3532-50 LCR Hi Tester) at different frequency from 42 Hz to 5 MHz at room temperature. The electrical study for samples showed that all samples are semiconductor materials. The hind wing showed the largest conductivity compared to the other samples (Fig. 5).

2. Voltage Measurement.

The Elytron of the Adult Insect. When measuring the voltage of the elytron to the adult insect from the dorsal and lower side it gives a reading of ~0.96 mV, and by repeating the measurement many times we discovered that there is no voltage discharge of the surface of the elytron.

By exposing the dorsal and ventral sides of the wing to an external light source [LED (CSA-TR-64/90°C - 26AWG/LL85683) for 1 min at 14 v - 0.15 A] no effect on the voltage readings of the elytron was noticed.

The Hind Wing of the Adult Insect.

1) At the beginning of measuring the voltage of the dorsal side of the hind wing of the adult insect gave an average voltage reading of ~55 mV, and the lower side gave an average voltage reading of ~75 mV. This reading is much more than the measured on the elytron.
   These result values match with the EDAX analysis, where the percentage of the transition elements in the hind wing of the adult insect is higher than those materials in the elytron.
   The hind wing of the adult insect showed gradually voltage discharge till 2 mV by repeating the measurement many times on both dorsal and lower sides (Fig. 6).
2) By exposing the dorsal and lower sides of the wing to an external light source [LED (CSA-TR-64/90°C - 26AWG/LL85683) for one minute at 14 v - 0.15 A], the material of the hind wing showed sensitivity to the light, where the light acted as a voltage charger to the hind wing.
   When measuring the voltage of the hind wing after charging from exposure to the light, it gives a reading of ~59 mV, by repeating the measurement many times the hind wing showed gradually voltage discharge till (2 mV) (Fig. 7).

The Area between the Thorax and the Abdomen (Sterna).

1) From the outside: the sterna of the adult insect does not show any voltage reading.
2) From the inside: the sterna of the adult insect showed a high voltage reading ~77 mV and it discharged gradually till ~25 mV which appears to be the constant reading of this part by repeating the measurement many times (Fig. 8).
   - When measuring the voltage reading outside the body of the adult insect, it did not show any voltage reading which indicates that it is an insulator.
   - The sterna of the adult insect from inside showed a high voltage, and its gradually discharge indicates that there is a relation between it and the hind wing.

A semiconductor is a substance, usually a solid chemical element or compound that can conduct electricity under some conditions but not others, making it a good medium for the control of electrical current. Its conductance varies depending on the current or voltage applied to a control electrode, or on the intensity of irradiation by infrared (IR), visible light, ultraviolet (UV), or X rays.

The specific properties of a semiconductor depend on the impurities, or dopants, added to it. In a manner similar to the conduction of current in a wire, a P-type semiconductor carries current predominantly as electron deficiencies called holes. A hole has a positive electric charge, equal and opposite to the charge on an electron. In a semiconductor material, the flow of holes occurs in a direction opposite to the flow of electrons.

Silicon is used to create most semiconductors commercially. A pure semiconductor is often called an "intrinsic" semiconductor. The conductivity, or ability to conduct, of common semiconductor materials can be drastically changed by adding other elements, called "impurities" to the melted intrinsic material and then allowing the melt to solidify into a new and different crystal. This process is called "doping".

For the group IV semiconductors such as silicon, germanium, and silicon carbide, the most common dopants are acceptors from Group III or donors from Group V elements. Boron, arsenic, phosphorus, and occasionally gallium are used to dope silicon.

Gallium is used as a dopant for the production of solid-state devices such as transistors. However, worldwide the actual quantity used for this purpose is minute, since dopant levels are usually of the order of a few parts per million.

The insect elytron is a biopolymer that offers a variety of structures and additional junctions including elastic energy storage.

The development of biomemic materials suitable for industrial production may be assisted by the design principles identified in these biological tissues.

The surfaces of RPW wings contain tapered, papillae structures that are distributed in a quasi-periodic array.

The papillae found on RPW ventral elytron and the hind wings are believed to enhance the optical transmission through the surfaces by gradually matching the dielectric constants across the interface between the media.

EDAX of the elytron composed of: C, Cl, Fe, Cu and Re. These materials are of transient elements, EDEX of the hind wings clarified that the elements of hind wings are: Cu, C, N, K and Zn, transient elements.

EDAX of the hind wing of RPW without using gold, proved the presence of Si and P. Si is in the form of SiO_2 and P is in the form of GaP. This proves that hind wings behave as semiconductor.

Conductivity of the elytron and hind wings are in the field of semiconductors, hence the measurements of the conductivities were 6.63×10^{-7} and 4.16×10^{-9} respectively.

The measurements of volt of sterna and wings of RPW were high, this means that wings RPW behave as semiconductor and solar system.

In conclusion, it is clear that the elytron and hind wings of red palm weevil RPW contain compounds with transient elements and materials that have photoelectric electric effect as K. At the same time hind wings have Si in the form of SiO_2 and P in the form of GaP that have the behaviour of semiconductors.

This means that it is possible to biomemic these wings in the industrial system concerning semiconductor and solar system. This throws light on this area of research in order to biomemic these insects and transfer this vision to industrial area.

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Figures

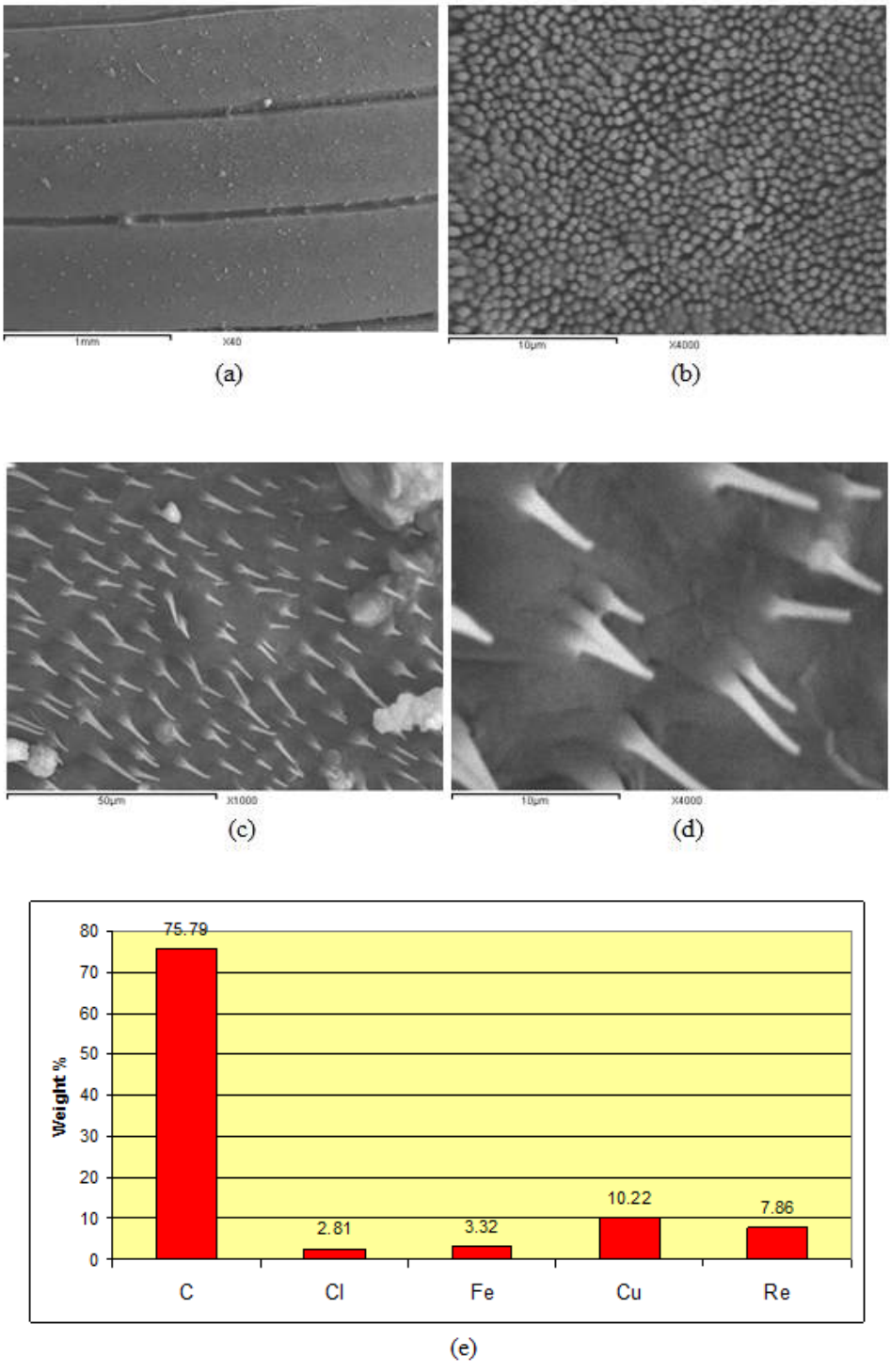


Fig. 1. a and b) dorsal surface of elytron; c, d) ventral surface of the elytron of RPW; e) EDAX of samples.

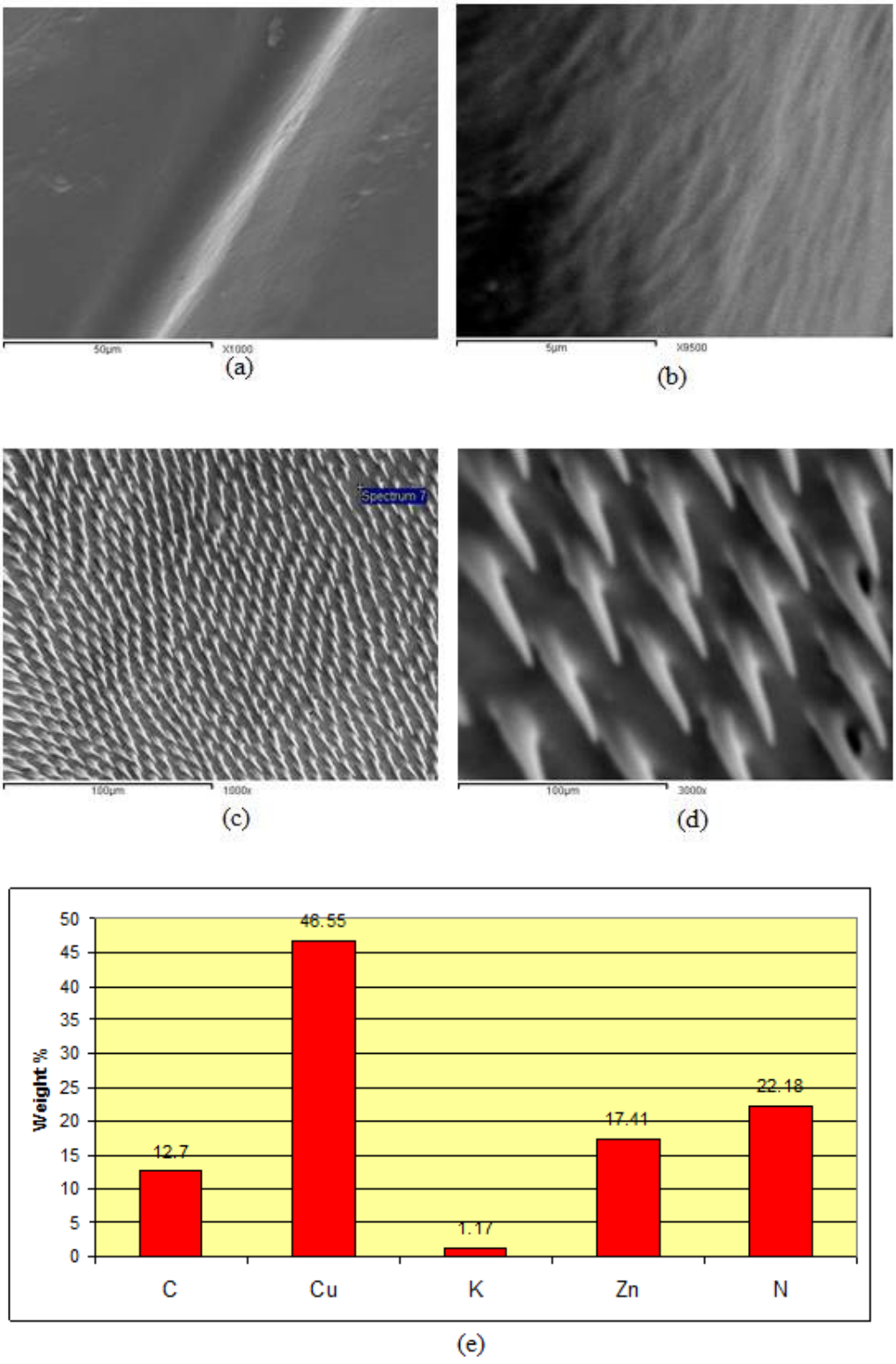
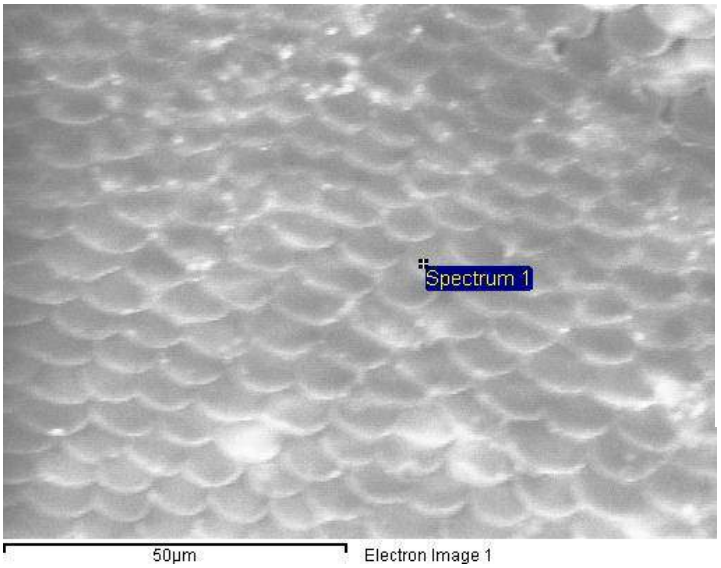


Fig. 2. a and b) dorsal surface of hind wing; c, d) ventral surface of the hind wing of RPW; e) EDAX of samples.



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No peaks omitted

Processing option : All elements analyzed (Normalised)
Number of iterations = 5

Standard :
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O SiO_2 1-Jun-1999 12:00 AM
Cl KCl 1-Jun-1999 12:00 AM
K MAD-10 Feldspar 1-Jun-1999 12:00 AM
Cu Cu 1-Jun-1999 12:00 AM

Element	Weight (%)	Atomic (%)
C K	70.92	77.04
O K	27.67	22.57
Cl K	0.32	0.12
K K	0.38	0.13
Cu K	0.71	0.15
Totals	100.00	

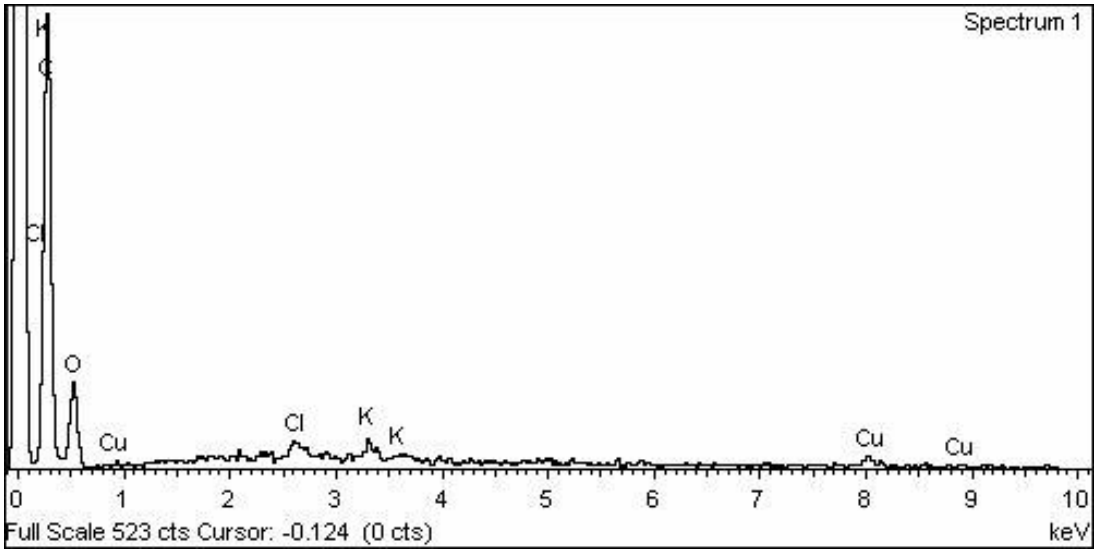
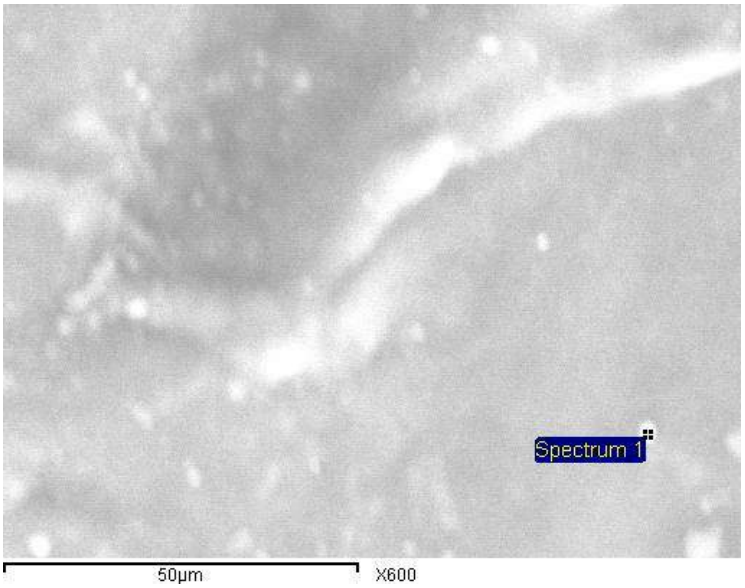


Fig. 3. The EDAX of the elytron without using gold as adhesive.



Spectrum processing :
No peaks omitted

Processing option : All elements analyzed (Normalised)
Number of iterations = 5

Standard :
C $CaCO_3$ 1-Jun-1999 12:00 AM
O SiO_2 1-Jun-1999 12:00 AM
Si SiO_2 1-Jun-1999 12:00 AM
P GaP 1-Jun-1999 12:00 AM
Ca Wollastonite 1-Jun-1999 12:00 AM
Cu Cu 1-Jun-1999 12:00 AM

Element	Weight (%)	Atomic (%)
C K	64.49	72.00
O K	32.02	26.84
Si K	0.12	0.06
P K	0.22	0.10
Ca K	2.80	0.94
Cu K	0.35	0.07
Totals	100.00	

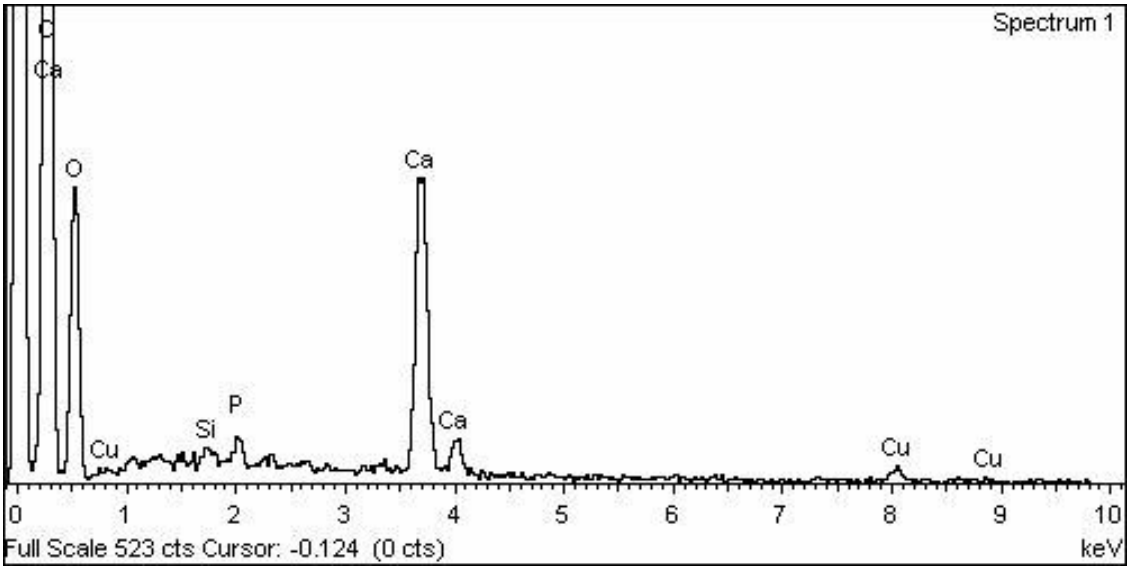
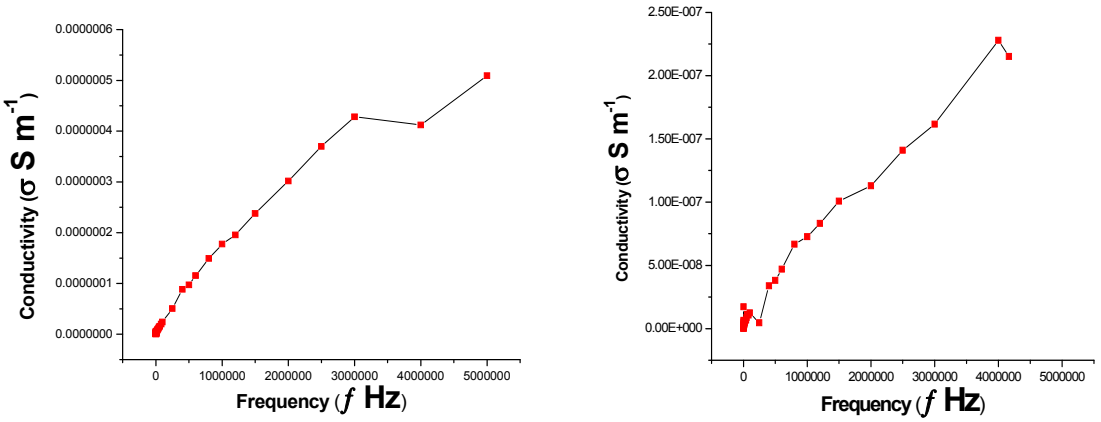
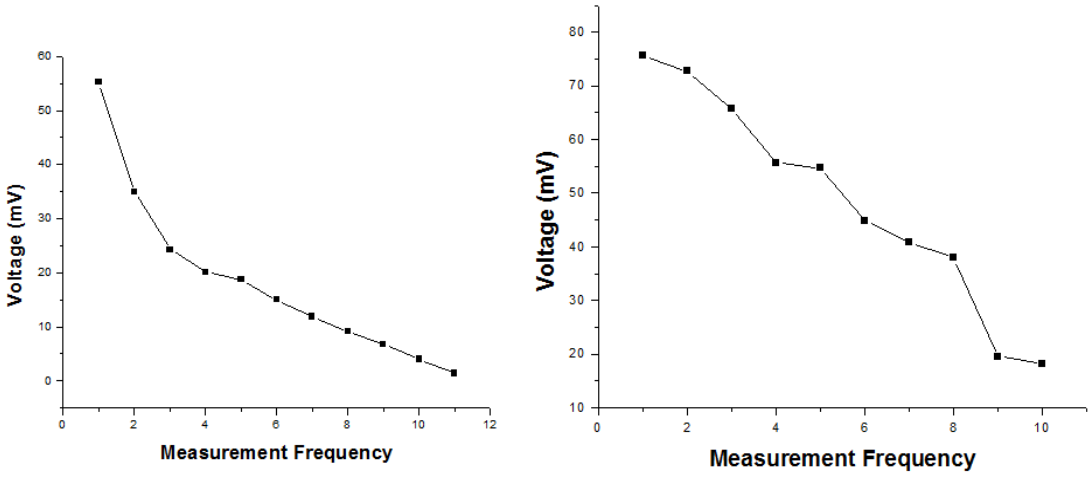


Fig. 4. The EDAX of the hind wing without using gold as adhesive.



(a) (b)

Fig. 5. Relation between frequency and conductivity for a) elytron and b) hind wing.



(a) (b)

Fig. 6. The hind wing readings a) dorsal and b) ventral.

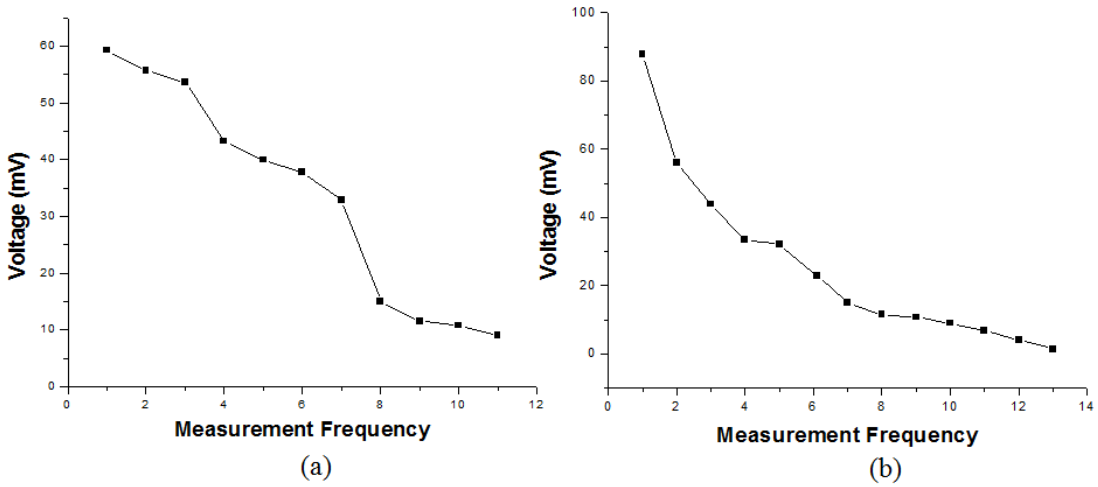


Fig. 7. The hind wing readings a) dorsal and b) ventral after applying light for one minute.

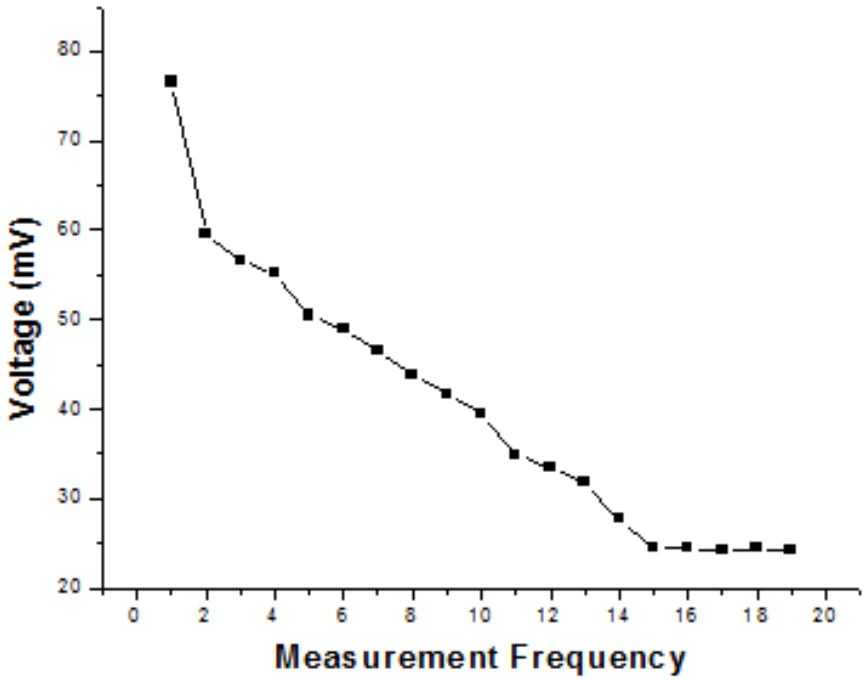


Fig. 8. The readings of the area between the thorax and body (dorsal view).

Collection, Identification and Application Potential Determination of Natural Enemies of *Batrachedra amydraula* Meyr.

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Iran

Keywords: biocontrol, *Batrachedra amydraula*, date palm, Iran, natural enemies

Abstract

The lesser date moth *Batrachedra amydraula* Meyr. is one of the most important pests of date fruits in hobabook and kimri stages in south of Iran, especially in the Bushehr and Khuzestan provinces. The larvae feed on embryonic tissues of immature date fruits and cuts the relation between pedicle and fruits, then dry fruits defoliate. Chemical control of this pest, not only was not successful, but also it causes ecosystem pollution and omits some of the predators, parasites and other useful animals. However, natural enemies of this pest were collected, identified and then the application potential of each was determined. Date fruits that were affected by these larvae were obtained weekly from 10 orchards of different zones, at the beginning of the pest activities from Dashtestan and Tangestan regions in Bushehr province. They were reproduced and regenerated in special containers at the laboratory. Two Hymenopter bees *Bracon* sp. and *Gonizus* sp. as parasitoids, one larval pathogen bacterium *Pasteuria* sp. and one spider of the family *Thomisidae* were identified. Their potential and parasitism were determined. The active parasitoids, predators and bacterial pathogen on lesser date moth (LDM) larvae showed a high potential of biological and natural pest control in Bushehr province with lowest chemical application and environmental pollution in the ecosystem.

INTRODUCTION

The lesser date moth (*Batrachedra amydraula* Meyr.) is one of the important and hazardous pests of date fruits in the southern date palm plantations of Iran, especially in Bushehr and Khuzestan provinces. This pest also is a problem in date palm growing areas of Iraq, Yemen, Palestine, Libya, Egypt and some other countries of the Middle East, in hobabook and kimri stages of date fruits (Gharib, 1967). The larvae feed on immature date fruits causing fruit quality and quantity reduction by cutting pedicle and then severing the fruit and defoliation. The activity of adult moths starts 10-15 days after pollination with feeding on pollen. Then the larvae feed on the young fruits. The larva digs hole on the end of fruits (connecting place of caps and pedicle), arrives at the fruit pit and feeds on it. The fruit-pedicle connection is cut and fruit defoliation will happen. During this process, the damaged fruits usually are reddish-dry, hence the native date growers call it "Homeira" or "Sorkheh" meaning red color pest. A larva may affect 1-5 fruits. The pest has 2-3 generations in 35-40 days' time of its life cycle. In the Bushehr province, beginning of the insect life cycle happens mid spring (April) until the end of the spring season (June). The third generation matured larvae pass the over summering and over wintering in the pupae phase (Gharib, 1973). The control measures of this pest generally include mechanical control methods such as date bunch covering after pollination and chemical methods such as aerial spraying, bunch surface terrestrial spraying and mix application of dusty chemicals with pollens during pollination are applied techniques for LDM control in Iran. In other cases, aerial spraying of chemicals by ULV method was carried out in Iraq and showed good effect on LDM (Al-Safi et al., 1994), but this method was not applied, because it causes environmental pollution. The hanged strips on each bunch infested by fumigate insecticides such as Dichlorfos were very effective in reducing the LDM damages in occupied Palestine (Bluberg et al., 1977).

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In Bahrain and Yemen many chemicals were tested by terrestrial spraying method (Abdul Jabbar et al., 1982; Engod, 1987). In occupied Palestine, the insect growth regulator (IGR) compounds were applied against LDM and desirable results were obtained (Baiton, 1985). In Iraq, application of dry powdery formulations of three insecticides, Fenitrothion, Chlorpyrifos and Pyrimifos methyl contemporarily with pollination on date bunches, showed a high reducing effect on LDM population (Al-Samerraei et al., 1988). This method was carried out in an applied research design by five kinds of powdery insecticides including Fenitrothion, Diazinon, Malathion, Chlorpyrifos and Pyrimifos methyl, mixed in pollens contemporarily with date palms pollination against the LDM in Iran and desirable results were reported (Karampour and Afshari, 1998, 2000). The special status of date palm plantations, especially in the old orchards such as high trees, wide flood channels and streams, shrubs growth and unlevel areas, make it difficult for a terrestrial chemical spray. Therefore both in Iran and the neighboring countries, aerial spraying was currently applied.

Because some problems such as long distances among orchards, pesticides solution drift, high temperature during spraying, very high airplane flight and date palm umbellate canopy which prevent receiving the pesticide solution to date bunches and pest larvae, the aerial spraying has low effect and causes environmental pollution. Finally the high amount of time and costs of this method make its uneconomic and not applicable to control the pest. In fact many applied research and studies showed that this method is not a suitable way for LDM control in Iran (Afshari and Kajbaf Vala, 1996). Therefore we tried to find safe integrated management control measures and a combination of mechanical, chemical and biological methods for date palm pest control.

Some parasites such as *Bracon brevicornis* Wesm. *Microbracon hebetor* Say. and *Phanerotoma aculari* Koh. were reported in date palm plantations of Khuzestan province in Iran (Gharib, 1967) and Basra province in Iraq (Hussein, 1989). Also *Gonizus* sp. was reported in Khuzestan as an ectoparasite of LDM larvae (Kajbaf Vala, 1994). In addition to *Bracon* and *Gonizus* as two active parasites, we collected an arachnid predator species of the family *Thomisidae* and a bacterial pathogen species *Bacillus* (*Pasteuria*) sp. on LDM larvae in Bushehr province, and evaluated the efficiency of all these natural enemies of LDM (*Batrachedra amydraula* Meyr.) in Iran for the first time.

MATERIALS AND METHODS

At the beginning of LDM activation in date palm plantations, collection and identification of its natural enemies was carried out. For this objective, 10 infected orchards per each region including Dashtestan and Tangestan date plantations regions were selected. Observations and sampling was done weekly. The samples were collected among immature infected fruits in hobabook and kimri stages. They were put in plastic bags separately; time and place of collection labeled and transferred to the laboratory. The sampled larvae, eggs, pupae and adult insects were stabilized under the special containers for detection of exiting natural enemies in the laboratory. All of the specimens were observed and identified by binocular and objective loops and their parasiting was studied very carefully. On the other hand stabilized light traps in the date palm orchards collected active arthropod predators such as arachnids and probable predatory activities were studied.

The active parasites, predators and pathogens, were purified and identified by scientific special keys, in the insects' taxonomy department and biological control research department of Iranian plant pests and diseases research institute (PPDRI). The infected larvae by bacterial agents, showed a slimy layer and soft rot on the larvae bodies. The surface of infected larva were disinfected by sodium hypochlorite (10% Clorox) and washed by sterile distilled water. Then the external skin of infected specimens was split by pances, scissors and scalpels in clean and disinfected conditions. Internal infected tissues of the insect body were separated under stereomicroscope field observation. A piece of internal tissue was diluted in the ratio of l:10 sterilized distilled water and a drop of the last dilution which contained the lowest population of bacteria was cultured on

nutrient agar medium and water agar under the microbiological laminar flow sterilized by ultra violet-ray and ethanol. The purified colonies of bacterium were obtained and these bacteria were incubated to few healthy larvae again. After one week, symptoms of disease as slimy cover and soft rot were observed on larva body and larvae died after 10 days. Also, to study parasiting nematodes and fungi activation, all of LDM life cycle stages (eggs, larvae, pupae and the adult moths) especially larval stage, macroscopic and microscopic studies were done carefully, but no symptoms and/or signs were observed. To determine the application potential of isolated parasites and pathogenic bacterium, in 4 weeks' time from the beginning of April, infected larvae were collected from 10 date palm trees of each 10 selected orchards (Total: 10×10=100 trees) per week and they were counted statistically. For all of the parasitized and diseased larvae, parasitism percentage and pathogenicity percentage in comparison to total population of pest in each statistical sample, were counted and determined. Then all data were registered in special tables.

RESULTS AND DISCUSSION

In many cases of established light traps in orchards and also in some samples, the arthropods such as many active spiders belonging to the family *Thomisidae* were isolated on LDM adult moths. In many specimens of LDM larvae a kind of soft rot disease that showed a dirty slimy cover on larva skin were obtained and a bacterium was isolated from them (Tables 1-4). After isolation and purification, based on morphological characteristics and laboratorial tests, this agent was identified as *Pasteuria* (*Bacillus*) sp. The pathogenicity test of this agent on LDM larva was positive and confirmed by scientific centers.

Also, in many specimens there were active larvae of two parasitoids, feeding on internal tissues of the LDM larvae and generally coming out from the dorsal side of them. They were identified as *Bracon* sp. and *Gonizus* sp. and of which the *Bracon* population was larger than *Gonizus* on LDM larvae (Tables 1-4).

Based on statistical analysis of data, following results obtained; the pest population of the larval stage increased from the first week to the third week of April, then it was demonstrated and after few days, the population decreased (Tables 1-4). The peak of larvae activation and damages in the ten sampling zones occurred at the third week of April (Table 3). The parasitism of larvae by *Bracon* sp. and *Gonizus* sp. happened from the first week (21.7%), increased to 22% at the second week and the peak of parasitism occurred at the third week (27%) in April (Tables 1-4). At the fourth week of April, in dry warm weather with a temperature up to 35°C and decreased relative humidity, parasitism came low gradually (Fig. 2). There were no pathogenic and infectious diseases on LDM larvae in specimens at the two first weeks of April, but infectious bacterial soft rot and slimy covering was observed on numbers of larvae at the third and fourth week more and less, especially in humid regions and date palm orchards with a flooding irrigation method (Tables 3 and 4). The most infected diseased larvae were observed at the last two weeks. Although bacterial disease in natural conditions of date plantations happened, it was not excessively. The predator spiders of the family *Thomisidae* and some of the other spiders on LDM and the other date palm pests such as *Ommattissus binotatus* were observed frequently on the date trees in samples. They are very active and generally useful in biocontrol of many date palm pests.

In accordance to the results of this research, there are a few applied recommendations which all date growers must know and could apply as management concepts to date pests' control:

1) The biocontrolling bees such as *Bracon* sp. and *Gonizus* sp. respectively act as two active and applicable natural enemies of the LDM larvae in the natural conditions of date palm plantations of Bushehr province and generally south of Iran. Therefore support, protection, reproduction, nourishment and releasing them at suitable times (beginning until the end of April) may be very useful in biological control of LDM and can cause a population reduction of more than 25%, thus it causes a decrease in damage.

2) The bacterial soft rot disease of LDM larval stages caused by *Bacillus* sp. in April especially in high relative humidity and moderate temperature conditions can kill almost 5.8% of larvae and in fact decrease damage.
3) The date palm plantations have usually a high potential of predators such as spiders which are very active in biocontrol of important and hazardous pests such as LDM and *O. binotatus*. We should protect and train them to support natural control measures.
4) There are alive and active populations of useful animals, prokaryotes and fungi in date plantations of which a few are known and also many are unknown to us surely. We should protect them from pesticides, fungicides and other general chemicals, which may kill them. We must protect our clean and safe ecosystems, especially in date orchards to health and survival of the human and the other living creatures. We can have safe crops and fruits without any poisonous residue if we want.

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Tables

Table 1. Parasitism and disease severity in first sampling (1st week April).

Orchard No.	Total larvae (number)	No infected larvae (number)	Parasitized larvae (number)	Parasitism (%)*	Diseased larvae (number)	Disease (%)
1	12	9	3	25.00	-	-
2	7	6	1	14.28	-	-
3	9	8	1	11.11	-	-
4	13	9	4	30.77	-	-
5	8	6	2	25.00		-
6	4	3	1	25.00		-
7	11	8	3	27.27		-
8	7	14	3	17.65		-
9	16	13	3	18.75		-
10	4	3	1	25.00		-
Total	101	79	22	-		-
Mean	10.1	7.9	2.2	21.78		-

* This relation determined the larvae parasitism percentage:

$$PPL = \frac{NPL}{TNL} \times 100$$

NPL=number of parasitized larvae
TNL=total number of larvae
PPL= parasitism percentage of larvae

Table 2. Parasitism and disease severity in second sampling (2nd week April).

Orchard No.	Total larvae (number)	No infected larvae (number)	Parasitized larvae (number)	Parasitism (%)	Diseased larvae (number)	Disease (%)
1	18	13	5	27.78	-	-
2	16	13	3	18.75	-	-
3	19	15	4	21.05	-	-
4	24	18	6	25.00	-	-
5	13	10	3	23.07	-	-
6	7	6	1	14.28	-	-
7	21	16	5	23.81	-	-
8	27	22	5	18.52	-	-
9	19	14	5	26.32	-	-
10	5	4	1	20.00	-	-
Total	172	131	38	-	-	-
Mean	17.2	13.1	3.8	22.09		-

Table 3. Parasitism and disease severity in third sampling (3rd week April).

Orchard No	Total larvae (number)	No infected larvae (number)	Parasitized larvae (number)	Parasitism (%)	Diseased larvae (number)	Disease (%)*
1	28	19	7	25.00	2	7.14
2	22	15	6	27.27	1	4.54
3	25	20	5	20.00	-	-
4	33	22	9	27.27	2	6.06
5	19	15	4	21.05	-	-
6	9	6	3	33.33	-	-
7	34	22	9	26.27	3	8.82
8	39	27	11	28.21	1	2.56
9	27	8	9	33.33	-	-
10	7	5	2	28.27	-	-
Total	243	169	62	-	9	-
Mean	24.3	16.9	6.5	27.05	1.8	5.82

* This relation determined the larvae disease percentage:

$$PPL = \frac{NDL}{TNL} \times 100$$

PDL=percentage of diseased larvae
NDL=number of diseased larvae
TNL=total number of larvae

Table 4. Parasitism and disease severity in fourth sampling (4th week April).

Orchard No	Total larvae (number)	No infected larvae (number)	Parasitized larvae (number)	Parasitism (%)	Diseased larvae (number)	Disease (%)
1	26	18	6	23.08	2	7.29
2	20	14	6	30.00	-	-
3	26	19	5	19.23	2	7.69
4	34	25	8	23.53	1	2.94
5	17	14	3	17.65	-	-
6	2	13	7	33.33	1	4.76
7	31	23	6	19.33	2	6.45
8	26	25	9	25.00	2	5.56
9	23	16	7	30.43	-	-
10	16	11	5	31.25	-	-
Total	250	178	62	-	10	-
Mean	25	17.8	6.2	25.29	1.67	-

Figures

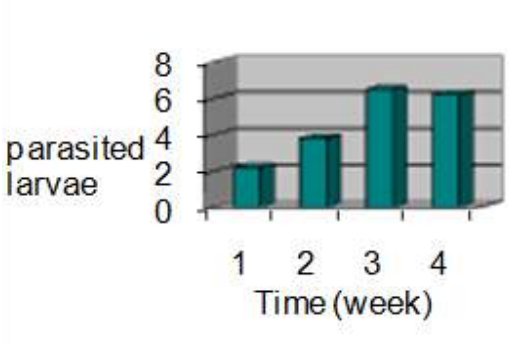

Fig. 1. Population changes in parasite larvae

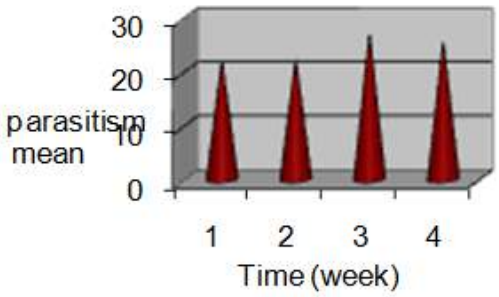

Fig. 2. Relative parasitism percentage larvae.

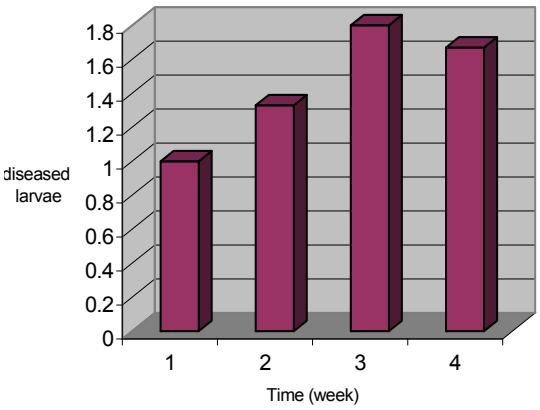

Fig. 3. Population changes in diseased larvae.

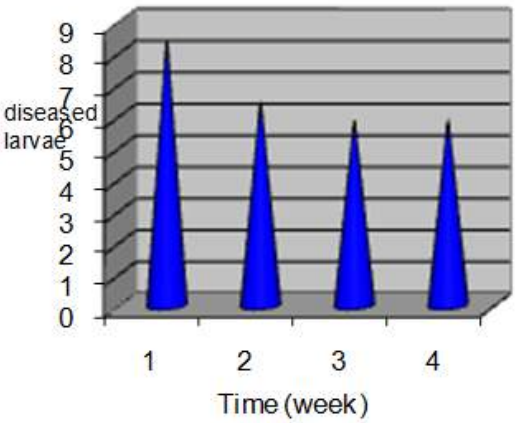

Fig. 4. Relative diseased larvae.

Biophysical Properties of Date Paste: Assessment of Flow Behavior and Texture

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Keywords: date paste, textural profile analysis, forward extrusion, Casson model, flow behavior, Ottawa test

Abstract

The rheological properties of two commercial date pastes were investigated in the temperature range of 20-70°C. From typical flow behavior curves, it was observed that date pastes exhibited pseudoplastic behavior. The shear stress-shear rate data were fitted using six common rheological models. The Casson model best described the experimental data at all temperatures. The Arrhenius model described successfully the temperature dependence of apparent viscosity of date pastes (R^2>0.99) with an E_a value in the range of 25392.6-25485.7 kJ/kmol. The textural attributes measured were: hardness, springiness, gumminess, cohesiveness, chewiness, adhesiveness for TPA test and firmness, adhesive force, mean load and total positive area for Ottawa test. There was a significant difference in textural attributes between two varieties studied. TPA results showed that all parameters obtained for Black date pastes were higher than Golden date pastes except for springiness and cohesiveness. However, the Ottawa results showed that Golden date pastes were firmer and less adhesive than Black date pastes.

INTRODUCTION

Among the agricultural commodities of commercial importance in Iran, dates are of particular interest. The date fruit is marketed all over the world as a high value confectionary and fruit crop. Today, there is a new interest in the date as a component of new food formulations/preparations (Ahmed and Ramaswamy, 2006). Date processing industries are producing various date products like date paste, date syrup, date dip, date honey, date jam, date vinegar, etc. (Ahmed et al., 2005). Date is generally steamed, de-stoned, macerated, and converted to a semi-solid form known as paste with approximately 20-23% moisture content and a water activity below 0.6 (Ahmed et al., 2005). Date paste has been used as filler and also sugar substitute in many food formulations. Confectionary industries have been utilizing date paste as one of the major ingredients. The possibility of using date paste as a replacement for caramel or sugar paste in preparing candy bars was studied (Yousif et al., 1991).

To assess the quality of semi-solid food products, it is necessary to understand their rheological properties. Rheological properties of foods are of great importance for several reasons especially in sensory evaluation, quality control, process design of flow equipments (mixing, pumping, heating, cooling, filling, etc.), new product development, process scale-up, and optimization of process variables (Abdelrahim et al., 1994; Steffe, 1996).

Texture is the most important factor for determining the overall quality and consumer acceptability of fresh fruits and vegetables. The texture profile analysis (TPA) technique has been used to characterize textural attributes of solid foods from empirical to

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instrumental results. The most notable early development in this area was made by Szczesniak et al. (1963), in which they attempted to classify textural properties, for example hardness, adhesiveness, cohesiveness and elastic quality, and propose scientific methods for their measurement.

Rheological properties of date pulp (15 and 45°Brix) and concentrates (73°Brix) were analyzed by El-Samahy et al. (2004). Physico-chemical properties of date pastes were investigated by Ahmed and Ramaswamy (2006). Instrumental texture profile analysis of date flesh as a function of moisture content was studied by Rahman and Al-Farsi (2005). The objectives of the present work were to evaluate comprehensively the rheological properties of two famous commercial Iranian date pastes. In this paper, the textural quality attributes of date paste investigated and flow behavior properties and temperature dependency were studied by steady shear viscometry. Meanwhile, the shear stress-shear rate data were fitted to six famous rheological models in order to select the best model describing the flow behavior of date paste.

MATERIALS AND METHODS

Materials

Two date fruits cultivars namely 'Mordab Sang' from Kerman province and 'Maktoom' from Fars province of Iran were harvested and their pastes produced by the Saberi factory in Mashhad, Iran. The trademark names of these date pastes were Golden and Black date pastes, respectively. Total soluble solids was measured with a Refractometer (Atago Com, Korea) and total solids by vacuum drying methods (70°C, 25 mm Hg and for 48 h). Samples were also analyzed for pH with a pH meter (Jenway 3020, UK). The average chemical characteristics of date paste samples used in this study are summarized in Table 1.

Texture Profile Analysis (TPA)

For two-cycle compression, a texture analyzer QTS (CNS Farnell Company, UK) was used to measure the TPA of date pastes. Samples of date paste were placed carefully on the metallic surface of the texture analyzer. Then the cross-head was allowed to penetrate with a cylindrical probe (25 mm diameter) at the rate of 30 mm/min to a total deformation 20 mm and back to original position followed by second down and up cycle on the same sample. Six replicates were conducted for each date paste sample and all operations were automatically controlled by the texture analyzer. The following parameters were extracted from the generalized instrumental texture profile curve: hardness cycle 1, gumminess, chewiness, adhesiveness, springiness, cohesiveness, adhesive force, hardness cycle 2, area cycle 1 and area cycle 2. Hardness is defined as the force necessary to attain a given deformation or a penetration in a product (Szczeniak et al., 1963). Area cycle 1 (A_1) is the total energy required for the first compression and area cycle 2 (A_2) is the total energy required for the second compression. Cohesiveness (A_2/A_1) is the extent to which a material can be deformed before it ruptures (Szczesniak, 2002). Springiness (or elasticity) is the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed (Szczeniak et al., 1963). Adhesiveness is the work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials with which the food comes into contact (Szczesniak, 2002). Chewiness is the energy required to masticate a solid food to a state ready for swallowing, or a product of hardness, cohesiveness and springiness (Szczesniak, 2002). Gumminess is the energy required to disintegrate a semi-solid food to a state ready for swallowing, or a product of a low degree of hardness and a high degree of cohesiveness (Szczesniak, 2002).

Forward Extrusion Test (Ottawa Cell)

The Ottawa cell is designed to produce shear stresses in a specimen by forward extrusion. As the plunger of the Ottawa cell is moved down the food specimen is

compressed. As deformation continues, the food is extruded through an insert in the bottom of the cell. This technique is ideally suited for testing beans, fruit fillings, pastes, soft vegetables, mashed potato and snack products. The Ottawa test jig consists of a square test cell with solid walls and an open base which can be fitted with a plate. A square plunger (43×43 mm) is fitted to the machine crosshead and provides the compression. The test method involves weighing a suitable quantity of sample which is placed in the cell with a slotted plate fitted (83×4 mm Ø holes). The result is determined by measuring the resulting force required to extrude the sample. The cross-head was allowed to penetrate at the rate of 30 mm/min to a total deformation 40 mm and back to original situation. The mechanical parameters of this test such as firmness, adhesive force, mean load and total positive area (area cycle 1) were extracted by texture analyzer software from the load-time and load-deformation curves. The firmness is defined as the force required extruding the specimen through the insert to a specified extrusion distance. Adhesive force is maximum negative force generated during upstroke of probe. Total positive area is positive area to firmness.

Flow Properties Measurements

Rheological parameters of date pastes were measured by a Bohlin Visco 88 viscometer (Bohlin instrument, UK) equipped with a cone and plate geometry (cone diameter 30 mm, cone angle 5°) and a heating circulator (Julabo, Model F12-MC, Julabo Labortechnik, Germany). The flow curves of date pastes were determined at four temperature levels of 20, 40, 60 and 70°C by increasing the shear rate from 14 to 500 s^{-1}. The shear rate increased up to 500 s^{-1} for Golden date paste and up to 60 s^{-1} for the other one. The shear stress-shear rate data were then fitted using six famous models known as Power law (Eq. 1), Bingham plastic (Eq. 2), Herschel-Bulkley (Eq. 3), Casson (Eq. 4), Sisko (Eq. 5) and Vocaclo (Eq. 6). To select the best model describing time-independent rheological properties of date paste samples, the coefficient of determination (R^2) was determined for the aforementioned rheological models. There is considerable evidence that the influence of temperature on viscosity, for a semi-solid food, may be described by an Arrhenius-type relationship (Eq. 7)

$$\tau = k\dot{\gamma}^{n} \quad (1)$$

$$\tau = \tau_0 + \eta\dot{\gamma} \quad (2)$$

$$\tau = \tau_0 + k\dot{\gamma}^{n} \quad (3)$$

$$\sqrt{\tau} = \sqrt{\tau}_0 + k\sqrt{\dot{\gamma}} \quad (4)$$

$$\eta = \eta_\infty + k\dot{\gamma}^{n-1} \quad (5)$$

$$\tau = \left[k\dot{\gamma} + (\tau_0)^{\frac{1}{n}} \right]^n \quad (6)$$

$$\eta = \eta_0 \exp(E_a / RT) \quad (7)$$

where, τ is the shear stress (Pa), τ_o is the yield stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency coefficient ($Pa.s^n$), n is the flow behavior index (dimensionless), η is the apparent viscosity (Pa.s), η_∞ is the viscosity at high shear rate (Pa.s), η_0 is the proportionality constant or viscosity at infinite temperature (Pa.s), E_a is the activation energy (kJ/kmol) and T is the absolute temperature (K), and R is the gas constant (kJ/Kmol.K).

RESULTS AND DISCUSSIONS

Texture Profile Analysis

Texture Profile analysis of date pastes is shown in Table 2. Hardness cycles 1 and 2, gumminess, cohesiveness, springiness and area cycle 1 were not significant between date paste samples analyzed in this study. Among analyzed parameters adhesiveness, chewiness and area cycle 2 at $p<0.05$ and adhesive force at $p<0.01$ were significant. Cohesiveness, springiness, adhesive force, adhesiveness, area cycle 1 and 2 were higher for Golden date paste than Black date paste.

Rahman and Al-Farsi (2005) reported a significant different value for date flesh. However, cohesiveness and springiness values obtained here were very close to those evaluated by Ahmed and Ramaswamy (2006) for date pastes.

Forward Extrusion Analysis

Forward extrusion analysis of date pastes is shown in Table 3. It can be found that the firmness and total positive area were different between two varieties ($p<0.01$). Firmness, adhesive force and total positive area were higher for Golden date paste than Black date paste. These data confirm TPA results obtained.

Flow Behavior

Fluid rheological parameters of date pastes were measured at different temperatures and the shear stress-shear rate data of date pastes were tested for various rheological models (Table 4). It can be found that Sisko and Vocadlo models did not fit at all the rheological data for two date pastes at different temperatures. Bingham and Power law models fitted on the flow behavior data adequately at lower temperatures and failed at higher temperature. The Herschel-Bulkley model was not suitable for Black date paste at all temperatures and for Golden at higher temperatures either, while the Casson model fitted well the shear stress-shear rate data obtained for both date pastes at each temperature (Table 4).

The apparent viscosity values of two date pastes at different shear rates are shown in Figures 1 and 2 as a function of temperature. These figures confirm the shear thinning behavior of date pastes at selected temperatures. At low shear rate, the apparent viscosity was varied significantly in the temperature range of 20-70°C for Golden date paste (Fig. 1), however, at shear rates higher than 300 s^{-1}, the viscosity did not decrease with shear rate and was almost constant.

Apparent viscosity decreased with increase in temperature. For Golden paste, yield stress (τ_0) decreased in the Casson model by increasing temperature, but such kind of behavior was not seen for Black date paste. Increasing temperature decreased Bingham yield stress (τ_0) and Bingham apparent viscosity (η) for the two date pastes.

El-Samahy et al. (2004) analysed the rheological properties of date pulp and date concentrate. They reported that date pulp is a pseudoplastic fluid and showed that the consistency coefficient "k" increases exponentially with increasing the soluble solids of the juice and it decreases sharply at higher temperatures. Temperature had a relatively small effect on the flow behavior index (n) of date pulp samples. They also concluded that the rheological parameters of date concentrate (k and n) gradually decreased by increasing temperature.

Figures 3 and 4 show the Arrhenius type behavior between the experimental apparent viscosity of Golden and Black date pastes and the inverse absolute temperature, respectively. Activation energy (E_a) indicates the sensitivity of the viscosity to the temperature change. The activation energy of Golden and Black date pastes ranged between 25485.7 and 25392.6 kJ/kmol, respectively. While the corresponding proportionality constants (η_0) were 1.92×10^{-4} and 1.002×10^{-3} Pa.s, respectively. The R^2 for both cases obtained 0.99. El-Samahy et al. (2004) determined activation energy for date pulp and concentrate and reported that as the total soluble solids of date pulps are increased the activation energy was increased. Date concentrates had higher activation energy compared with those for date pulp. Activation energies observed in this research were much higher than date pulp at 15 and 45°Brix and were lower than date concentrates reported by El-Samahy et al. (2004). The E_a and η_0 data obtained here were also higher

than those reported for tamarind juice concentrates (Ahmed et al., 2007).

CONCLUSION

Date paste showed a non-Newtonian, shear-thinning pseudoplastic, behavior, the relationship between viscosity and shear rate was non-linear. The viscosity decreased with increase of temperature. The model that best fitted the experimental data at all temperatures was the Casson model.

The textural attributes are significant parameters for semi-solid products such as date pastes. These parameters are very important for determining the overall quality and consumer acceptability of products. These parameters would help the process industries to design new food products, machineries and quality control. Cohesiveness, springiness, adhesive force, adhesiveness, area cycle 1 and 2 were higher for Golden date paste than Black date paste. Firmness, adhesive force and total positive area were higher for Golden date paste than Black date paste. These data may confirm TPA results obtained.

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Tables

Table 1. Chemical parameters of date pastes.

Parameters	Golden	Black
Total solids (%)	70.5±0.4	83.5±0.6
Total soluble solids (%)	60±0.4	70±0.6
pH	4.8±0.2	4.38±0.12

Values followed ± are the standard deviation.
Each data is the average of three replicates.

Table 2. Instrumental texture profile analysis (TPA) of date paste.

Texture parameters	Golden	Black
Hardness cycle 1 (g)	210.83±7.57	231.16±22.2
Adhesive force (g)	-101.83±11.3	-126.16±13.88
Gumminess (g)	158.50±8.69	172.23±13.33
Adhesiveness (gs)	-3135.06±513.71	-3860.25±447.39
Cohesiveness	0.75±0.04	0.74±0.06
Chewiness (g mm)	2841.74±341.29	2877.77±305.22
Springiness (mm)	17.89±1.51	16.68±0.68
Area cycle 1 (gs)	6372.84±350.17	5799.29±593.26
Area cycle 2 (gs)	4792.24±353.42	4321.58±369.15
Hardness cycle 2 (g)	204.33±9.75	216.16±16.62

Values followed ± are the standard deviation.
Each data is the average of six replicates.

Table 3. Forward extrusion analysis (Ottawa test) of date paste.

Extrusion parameters	Golden	Black
Firmness (g)	721.50±45.09	517.50±42.19
Adhesive force (g)	-425.25±34.85	-437.50±17.02
Mean load (g)	28.94±10.51	36.05±10.4
Total positive area (gs)	43950.00±3204.74	34071.54±1377.64

Values followed ± are the standard deviation.
Each data is the average of six replicates.

Table 4. Flow behavior parameters of date pastes obtained by some rheological models at different temperatures studied.

Date paste	Temperature (°C)	Bingham			Casson			Herschel-Bulkley				Power-law		
		τ_0	η	R^2	τ_0	k	R^2	τ_0	k	n	R^2	k	n	R^2
Golden	20	583.7	0.69	0.98	519.0	0.09	0.97	574.06	1.42	0.90	0.99	417.4	0.108	0.87
	40	408.1	0.19	0.77	417.5	0.01	0.63	259.5	1.34	0.81	0.72	448.1	0.055	0.58
	60	F	F	F	365.3	0.09	0.66	F	F	F	F	F	F	F
	70	F	F	F	270.6	0.19	078	F	F	F	F	F	F	F
Black	20	416.4	13.9	0.90	190.2	7.74	0.83	F	F	F	F	F	F	F
	40	363.5	5.16	0.88	267.4	1.53	0.90	F	F	F	F	215.0	0.38	0.93
	60	283.7	0.57	0.57	266.7	0.05	0.56	F	F	F	F	247.8	0.064	0.55
	70	F	F	F	307.3	0.18	0.66	F	F	F	F	192.8	0.09	0.58

F means failed the modeling of flow behavior data by the given rheological model.

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Figures

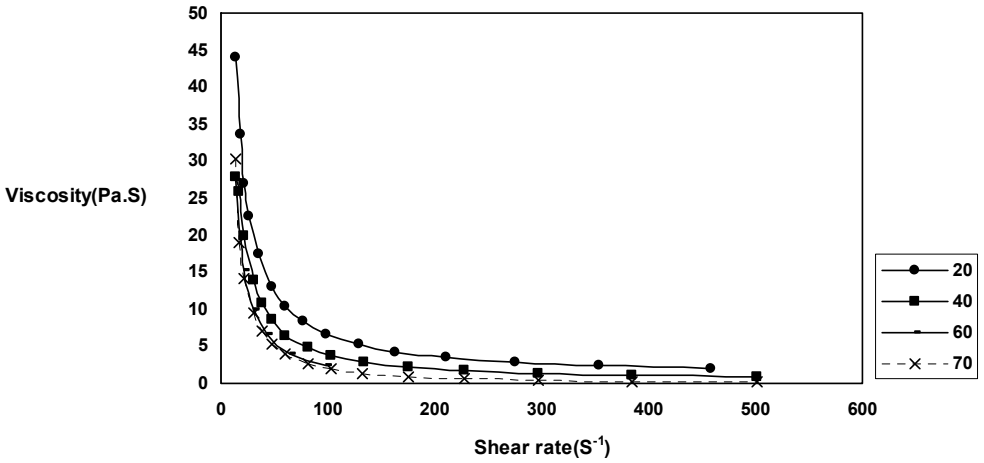

Fig. 1. Apparent viscosity - shear rate data of Golden date paste at selected temperatures.

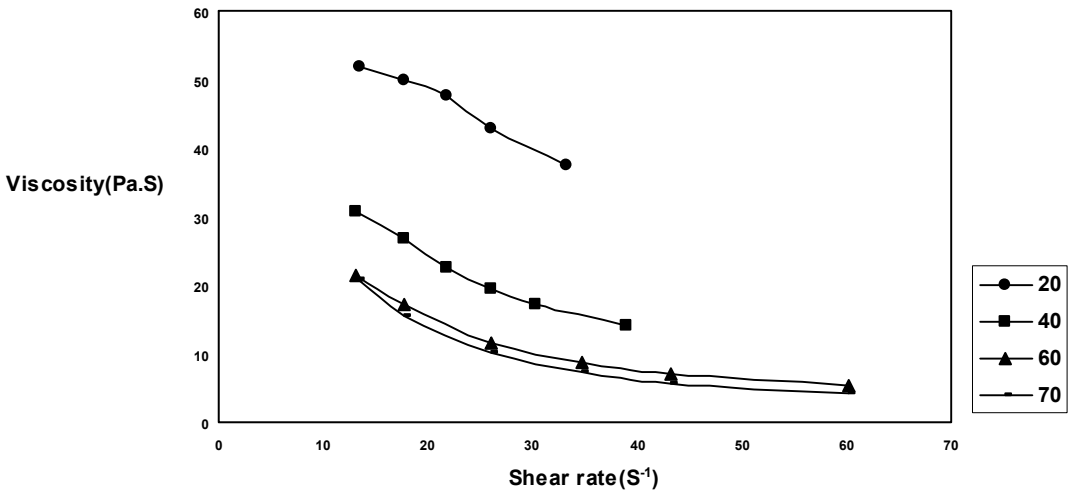

Fig. 2. Apparent viscosity - shear rate data of Black date paste at selected temperatures.

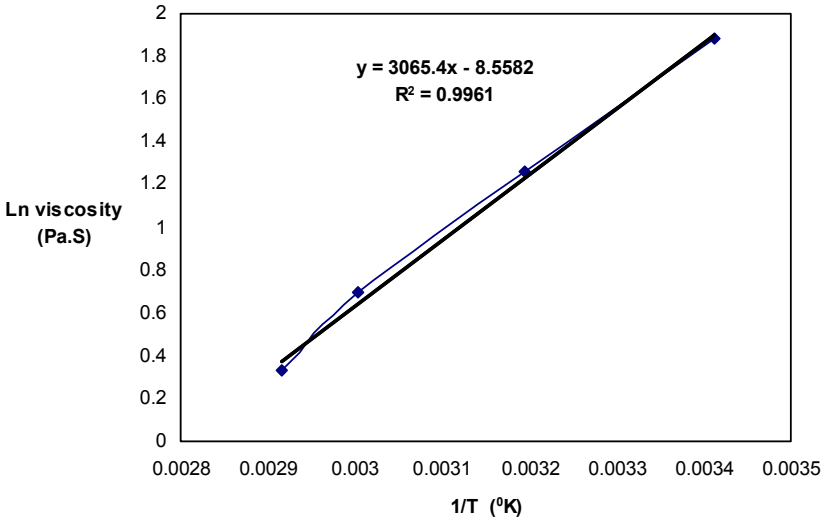

Fig. 3. The effect of temperature on the viscosity of Golden date Paste (E_a calculated for a temperature range of 20-70°C).

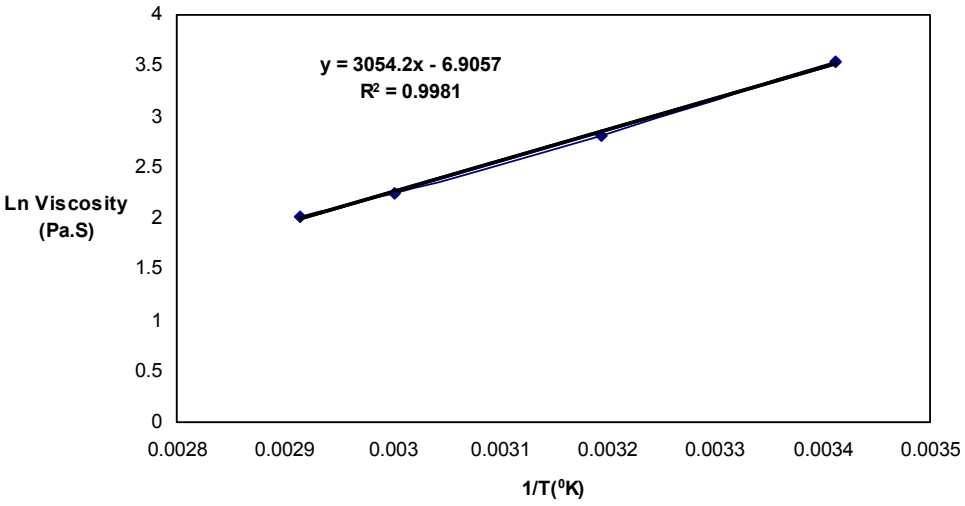

Fig. 4. The effect of temperature on the viscosity of Black date paste (E_a calculated for a temperature range of 20-70°C).

Modified Atmosphere Packaging of Date Fruit (*Phoenix dactylifera* L.) Cultivar 'Barhee' in Khalal Stage

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Keywords: date palm (*Phoenix dactylifera* L.), modified atmospheres packaging, quality attributes, storage, 'Barhee'

Abstract

Considering the date fruit (*Phoenix dactylifera* L.) 'Barhee' is mainly harvested at the khalal stage, the quality changes of fresh fruits were studied under modified atmosphere packaging (MAP). Fruit was packed in barrier bags and exposed to the ambient air (passive MAP) and different concentrations of CO_2 (5, 15 and 30%) within the packaging (active MAP) by using microperforated films as control. Fruits were stored at 4°C and physiochemical changes were studied at 7 days intervals during 28 days of storage. Modified atmosphere containing 30% CO_2 caused almost half of the fruits to turn into low quality rutab, while the best quality and longest shelf-life of khalal fruits was gained with 5% CO_2 concentration. Also, passive MAP compared with control samples, showed acceptable results by extending the shelf-life of khalal date fruits.

INTRODUCTION

Date (*Phoenix dactylifera* L.) is a berry fruit whose development divides into five stages namely hababook, kimri, khalal, rutab and tamar. At the khalal stage, fruits are physiologically mature, hard and crisp with over 50% moisture content, bright yellow or red in color and very perishable. In general, fruits at khalal stage are ready for commercial trade as "fresh" fruit but this applies only to those cultivars which are sweet, with a low amount of tannin and low astringency (Barreveld, 1993). Some date cultivars are suitable for marketing at the khalal stage including 'Barhee', 'Bereim', 'Hayany' and 'Khalas' among them 'Barhee' is the most popular cultivar worldwide (Mortazavi et al., 2007). The khalal fruits that are usually harvested at the end of July are highly perishable and must be transported to the market as soon as possible (Glasner, 2002). Any delay in transport or improper storage conditions result in quick appearing of rutab spots and surface wrinkling accompanied by a loss of flavor and taste (Mortazavi et al., 2007). The strategy for exporting khalal dates would make it necessary to develop new methods to delay fruit ripening during handling and storage. Modified atmosphere packaging (MAP) is now being used for extending the shelf-life and reducing the waste of a wide range of fruits and vegetables. There are few studies about use of vacuum and modified atmosphere packaging for date fruits at the khalal stage. Al-Redhaiman (2004) stored full mature 'Barhee' date fruits under three CO_2 concentrations (5, 10 or 20%) at 0°C and reported that fruits under 20% CO_2 have a statically longer storage period, lasting for 26 weeks. Also, Achour et al. (2003) determined that dehydrating of 'Deglet Nour' dates at tamar stage decreased at MA condition with 20% CO_2 and 80% N_2 during the storage period. A previous study by authors has demonstrated the potential interest of using passive MAP to reduce the weight loss and appearance of low quality rutab spots of 'Barhee' date picked at khalal stage. However, in the vacuum packaging, a large part of fruits turned to low quality rutab and fruit firmness reduced considerably (Mortazavi et al., 2007). The objective of this work was to study the influence of some active and passive MAP conditions accompanied by fruit to stalk junction status on the physiological properties, quality attributes and storability of 'Barhee' date fruits at the khalal stage.

MATERIALS AND METHODS

'Barhee' date fruits were harvested at khalal stage from a commercial orchard in Ahvaz, Khuzestan province, Iran according to yellow skin color and about 30% soluble solids concentration. The fruit were precooled immediately and transported to the laboratory. They were selected for no visual defects and basis of uniform size, color and without rutab spots, then washed with sodium hypochlorite solution 0.5% for 2 min, rinsed with tap water and dried prior to packaging.

Fruits were divided into eight lots each of 60 fruit, then each lot was divided into three replicates of 20 (200±5 g). Each set of three replicates was put into a dish tray and placed in a barrier nylon/polyethylene plastic bag (25×35 cm) given one of the eight treatments: (5%-J) joint fruits+5% O_2: 5% CO_2; (5%-D) detached fruits+5% O_2: 5% CO_2; (15%-J) joint fruits+5% O_2: 15% CO_2; (15%-D) detached fruits+5% O_2: 15% CO_2; (30%-J) joint fruits+5% O_2: 30% CO_2; (30%-D) detached fruits+5% O_2: 30% CO_2; (PM) detached fruits in passive MAP; and (C) control (detached fruits, perforated film providing ambient air atmosphere within the packages). All active MAP treatments (T1 to T6) were performed by creating a vacuum in a Henkelman vacuum pack instrument (200A) followed by flushing the gas mixture 1 bar pressure before heat sealing and N_2 used as a balance gas. All samples were stored at 4°C for up to 28 days. The experiment was conducted based on a completely randomized design (CRD). The data were analyzed with MSTAT-C (version 1.42) statistical package, and means compared by Duncan's Multiple Range Test (DMRT) at 0.01 and 0.05 probability levels. Visual examinations and other quality attributes were evaluated initially and periodically at seven day intervals. The gas atmosphere in the head space of the bags was analyzed using an O_2/CO_2 gas analyzer. The fruit subjected to all treatments were weighed before and after storage and data were expressed as percentage of weight loss. Fruit firmness was measured by a Wagner Penetrometer (FT 011). Titratable acidity was calculated as percentage of malic acid by titrating 10 g/100 ml of the date extract with a solution of NaOH (0.01 N) till pH 8.1. The pH was measured by a Metrohm pH meter. The level of sugars was measured as °Brix by an Atago refractometer. Water activity (a_w) values of fruits were measured at 30°C with a hygrometer. To determine the wrinkled area or rutab spots, to have more accuracy of data, an image processing procedure was used (Rocculi et al., 2005). Images were obtained, using a color plane scanner (true color-24 bit, resolution of 600×600 dpi), and by positioning the fruit halves on a scanner held on a black box, to exclude the surrounding light. The saved images were opened by a program that had been written by authors in Matlab® software to calculate the percentage of selected area fraction of fruit and averaged data were considered as RSA or WAP for each unit.

RESULTS AND DISCUSSION

During the storage period of 28 days, the fruit respiration resulted in a modification of the internal atmospheres and as expected, all treatments showed a reduced O_2 and an increased CO_2 level (Fig. 1). The oxygen concentration decreased sharply in the first seven days, from 5 to less than 2.7% and continued with a little decreasing amplitude in the following days. The final O_2 concentration was less than 0.07% for all treatments after 28 days storage. However the CO_2 production rate was higher at the first week for pouches containing 5% CO_2 (5%-J and 5%-D) but the final concentration of CO_2 was in keeping with its concentration at the start of the experiment. Clear correlation was observed between final and initial concentration of CO_2 and its concentration reached to about 60% for pouches containing 30% at the start of experiment. According to the O_2 curves, differences between passive and active MAP were seen during the transient period, then, drawing near and steady state was obtained after approximately 14 days of storage. These were in agreement with the results of Charles et al. (2008) for fresh endives.

It was evident from this study that the rutab spots area (RSA) that appeared during storage was correlated significantly with CO_2 concentration within pouches. The higher CO_2 concentration promoted RSA and image processing of fruit surfaces in 30%-J and 30%-D treatments (30% CO_2) showed 92.3 and 82.5% RSA respectively at the end of 28

days storage. Fruits in pouches filled with 15% CO_2, showed 49.5 and 58.8% rutab spots for 15%-J and 15%-D respectively and samples in 5% CO_2 condition (5%-J and 5%-D) undergoing a smaller turning to rutab spots. Also when the storage period prolonged, greater part of fruits changed to rutab in all treatments, particularly in fruits detached from stalk (5%-D, 15%-D and 30%-D). Fruits in the perforated bags exposed to the air (C), exerted highest WAP (24.25%) after 28 days of storage. WAP was negligible for other treatments and the lowest value was recorded in fruits that received 30% CO_2 (30%-J and 30%-D) at which a large part of fruits turned to rutab. Fruit to stalk junction had no major effectiveness on the wrinkled area percentage. Appearing rutab spots and wrinkled surface are the two main disorders restricting marketing, storage and exports of khalal dates after harvest (Fig. 2a,b). However, turning fruit surface color to brown considered as rutab spot, comparing obtained rutab fruits in this study, with those ripened naturally on the tree, showed some major differences. Rutab spots in the fruits coming out of storage can be described as CO_2 injury similar to that reported by Serrano et al. (2005), for sweet cherry at high CO_2 concentrations. Elevated CO_2 can prove to be fruit damage, often inducing fermentation, particularly when fruit is sealed in packaging film of insufficient permeability (Betts, 1996).

Regardless of gas composition within the package, fruit weight loss ranged between 1.13-1.82 g/100 g under MAP conditions, while control fruits in perforated bags (C) lost 10.48 g/100 g. The data revealed the pattern of reduction in fruit weight in all MAP packages was almost the same after 7, 14, 21 and 28 days of storage and packaging was effective in limiting weight loss of fruits (Table 1). As expected, the wrinkled area percentage (WAP) followed by a similar pattern with fruit weight loss (Weight loss = 0.525 WAP + 0.499). Weight loss is a physiological event caused by loss of water from the fruit surface to the surrounding atmosphere and loss of carbon on formation of CO_2 during respiration (Rizzo and Muratore, 2009). It can be controlled by temperature, humidity, and using proper packaging. In this work, for using barrier films, and minimum package condensation observed, it can be assumed that a gentle increase in weight loss of all treatments (except control), is caused mainly by the biochemical reactions of fruit cell components.

The initial firmness measured at the start of experiment was 3.4 kg. The following decrease in firmness during 28 days cold storage to 0.66 kg (average of all treatments) showed a clear response to the different MA conditions applied (Fig. 2c,d). Prolonging the storage duration, decreased the firmness of all samples gradually. The lowest flesh firmness was obtained in fruits stored under MA conditions with 30% CO_2 (30%-J and 30%-D). Date fruit at khalal stage had a hard and crisp texture and physico-chemical changes that caused arising rutab spots, decreased fruit firmness. Fruits in the pouches with 5% O_2 (5%-J and 5%-D) and control (C) showed the highest firmness (2.1, 1.9 and 2.2 kg respectively).

Significant differences between the treatments were found in terms of titratable acidity. TA level of date fruit in passive MAP (PM) and control (C) treatments were consistently lower than those of the other treatments. At harvest, the levels of total acidity (TA) calculated as malic acid was 22.48 mg/100 g fresh weight and TA increased significantly throughout the evaluation period for all samples (Fig. 2e,f). At the end of the experiment, pouches containing 30% CO_2 recorded nearly 1.5-1.7 fold higher acidity of the initial value. Also the results showed the fruits in pouches filled with 5% CO_2 had consistently lower acidity than those of treatments with 15 and 30% CO_2. However, detaching fruit from stalk, increased TA in 5 and 15% CO_2 treatments, but it did not show any significant effect on acidity levels. Similarly to rutab spots area, an increase in acidity level was apparent during storage time and direct correlation was observed among these studied parameters. Reversely, a gradual decrease in pH from 7.5 to 6.3 was seen during the 28 days of storage (Fig. 2e,f). Pouches filled with 30% CO_2 had maximum reduction in pH.

The pattern of changes in flesh softening, reducing pH and rising acidity level in fruits with high rutab spot area exhibit correlation with a high CO_2 level and it can be

concluded that the highest CO_2 level (30%) is not appropriate for storage of date fruits at khalal stage. This was nearly in contrast with the results of Al-Redhaiman (2004) that reported khalal date fruits have significantly longer storage period under higher CO_2 concentration.

Khalal fruits that lost their hard and crisp texture had lower quality and price. Softening of fruit texture is related to activation of pectin decomposing enzymes. The role of high CO_2 level in slowing texture softening and other enzymatic reactions has been underlined in different reports. Our results showed that CO_2 at 30% concentration had a negative effect on firmness due to stimulated fermentation, during that degradation of hemicellulosic polysaccharides and cellulose, leads to disorganization of cell wall, decrease in cellular turgidity and loss of texture firmness (Kader et al., 1989).

Increasing the acidity level upon storage and more specifically at the onset of deterioration of fruit texture postulated the second generation of organic acids formation (Barreveld, 1993). The accompanying increment of acidity level and brown surface discoloration of fruits also suggested that organic acids can react with reducing sugars to produce brown pigments (Lozano, 2006). Organic acids are a useful index of authenticity in fruits and have an important influence on the sensory properties of fruits especially in combination with sugars. Major organic acids that have been isolated from date flesh are citric, malic and oxalic acid (Barreveld, 1993). Fruit juice pH is affected by alkaline and acidic compounds of cells and any change in concentration of these compounds will change the pH quickly (Wills, 1998). Barreveld (1993) reported the most common pH values for 'Deglet Noor' date range from 5.3 to 6.3 and definite correlation was observed between increasing the pH and the commercial quality for this cultivar.

The water activity value in 'Barhee' date fruits at the beginning of the experiment was 0.97 (Fig. 2g,h). Evidently, after 28 days of storage, a slight decrease was observed in this parameter in all treatments but with different rates. The highest rate of a_w decline during the storage period occurred in fruits stored under MA with 30% CO_2 (0.94 in T5 and T6). On the contrary, the highest a_w value was observed in fruits stored under MA with 5% CO_2. A clear correlation was identified between decrease in water activity and rutab spots area, the less water activity, the higher Rutab spots (A_w = -0.0004 RSA + 0.9687). Water activity is the ratio of the partial vapor pressure of water in food to the partial saturation vapor pressure of water vapor in the air at the same temperature and describes the energy state of water in the food as an important quality factor for dates during storage (Fontana, 2000). Decreasing the level of a_w by storage duration revealed bonding more H_2O molecules to the solutes released from the fruit texture degradation.

As expected, significant changes in SSC were seen between different MAP conditions applied. Fruits exposed to 30% CO_2, displayed maximum amounts of SSC (33.2 and 32.9 for 30%-J and 30%-D respectively). Generally, most of the full mature khalal fruits gradually turned to low quality rutab fruits during storage. In all treatments by increasing the storage period, a greater part of fruits turned to rutab, and SSC increased from 27.7 to 34.3%. A direct correlation (SSC = 0.1039 RSA + 27.945) was observed between SSC and RSA. The lowest amounts of increase in the SSC and RSA were seen in pouches filled with 5% CO_2, passive MAP and control (31.4, 32.8, 34.1 and 33.6% respectively). SSC is one of the most important maturity and quality indices in various fruits and in date fruits at khalal stage, should be more than 28% (Barreveld, 1993). In contrast with produced rutab fruits in this work from khalal dates, the SSC content of rutab date fruits naturally ripened on the tree is about 70% and those have an acceptable quality. These findings are similar to those reported earlier by other workers on various date cultivars (Barreveld, 1993). It is postulated that the immobile form of the ripening enzymes existed in fruits harvested at khalal stage, is in contrast with dates left on the palm till they turn naturally to rutab. Formation of some metabolites including soluble contents arises by releasing and activating enzymes such as amylase, invertase, polygalacturonase and polyphenol oxidases (Saleem et al., 2005).

CONCLUSIONS

The date fruit of cultivar 'Barhee' lose their astringency at khalal stage and are sold as fresh fruit. This stage will last for a couple of weeks, terminating the supply for this purpose, leaving other fruits to mature further on the palm (Glasner et al., 2002). Fruit at khalal stage has a limited shelf-life and generally is sold shortly after harvest and mostly in the local markets. Based on our results we can conclude the significant improvement in postharvest storage of khalal 'Barhee' date fruits that can be achieved, under modified atmosphere conditions. Detrimental color changes of khalal dates were related to packaging conditions and, CO_2 injury was the direct result of the high CO_2 levels. A conservative recommendation to minimize quality losses would be to keep the khalal dates under 5% CO_2 level during storage. However, the best beneficial effects were obtained in active MAP, significant but not major differences were seen in passive MAP conditions and due to lower costs, it can be recommended when needed. Detaching fruit from stalk, showed negligible positive effects on maintaining the studied quality factors. Evident correlations were displayed between RSA and firmness, acidity, water activity and SSC postulated the biochemical changes of fruit issued from texture degradation by means of different enzymes. To understand better these biochemical changes, research dealing with and focused on the understanding of the processes involved might be proposed.

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Tables

Table 1. Weight loss modifications of different applied treatments during storage duration.

Storage duration (days)	0	7	14	21	28
5%-J	$0.00_{\pm 0.00}$	$0.45_{\pm 0.02}$	$0.62_{\pm 0.09}$	$0.94_{\pm 0.23}$	$1.58_{\pm 0.25}$
5%-D	$0.00_{\pm 0.00}$	$0.42_{\pm 0.03}$	$0.58_{\pm 0.01}$	$0.98_{\pm 0.19}$	$1.14_{\pm 0.12}$
15%-J	$0.00_{\pm 0.00}$	$0.42_{\pm 0.03}$	$0.61_{\pm 0.02}$	$0.73_{\pm 0.12}$	$1.82_{\pm 0.34}$
15%-D	$0.00_{\pm 0.00}$	$0.40_{\pm 0.04}$	$0.71_{\pm 0.13}$	$0.76_{\pm 0.07}$	$1.13_{\pm 0.23}$
30%-J	$0.00_{\pm 0.00}$	$0.39_{\pm 0.02}$	$0.68_{\pm 0.09}$	$0.74_{\pm 0.13}$	$1.28_{\pm 0.19}$
30%-D	$0.00_{\pm 0.00}$	$0.41_{\pm 0.01}$	$0.63_{\pm 0.06}$	$0.72_{\pm 0.05}$	$1.32_{\pm 0.57}$
PM	$0.00_{\pm 0.00}$	$0.39_{\pm 0.02}$	$0.60_{\pm 0.09}$	$0.81_{\pm 0.06}$	$1.23_{\pm 0.18}$
C	$0.00_{\pm 0.00}$	$3.17_{\pm 0.86}$	$5.50_{\pm 0.39}$	$8.91_{\pm 0.97}$	$10.48_{\pm 2.46}$

Figures

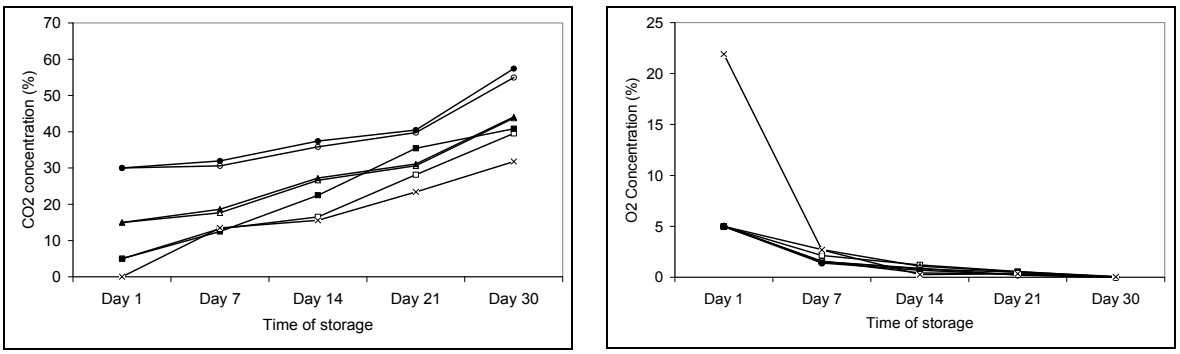

Fig. 1. Changes in CO_2 (left) and O_2 (right) partial pressure within pouches filled with 200±5 g of khalal date fruits as a function of time of storage, at 4°C, and packaging condition: (5%-J□) joint fruits+5% O_2: 5% CO_2; (5%-D■) detached fruits+5% O_2: 5% CO_2; (15%-J△) joint fruits+ 5% O_2: 15% CO_2; (15%-D▲) detached fruits+ 5% O_2: 15% CO_2; (30%-J○) joint fruits+5% O_2: 30% CO_2; (30%-D●) detached fruits+5% O_2: 30% CO_2 and (PM×) detached fruits in passive MAP.

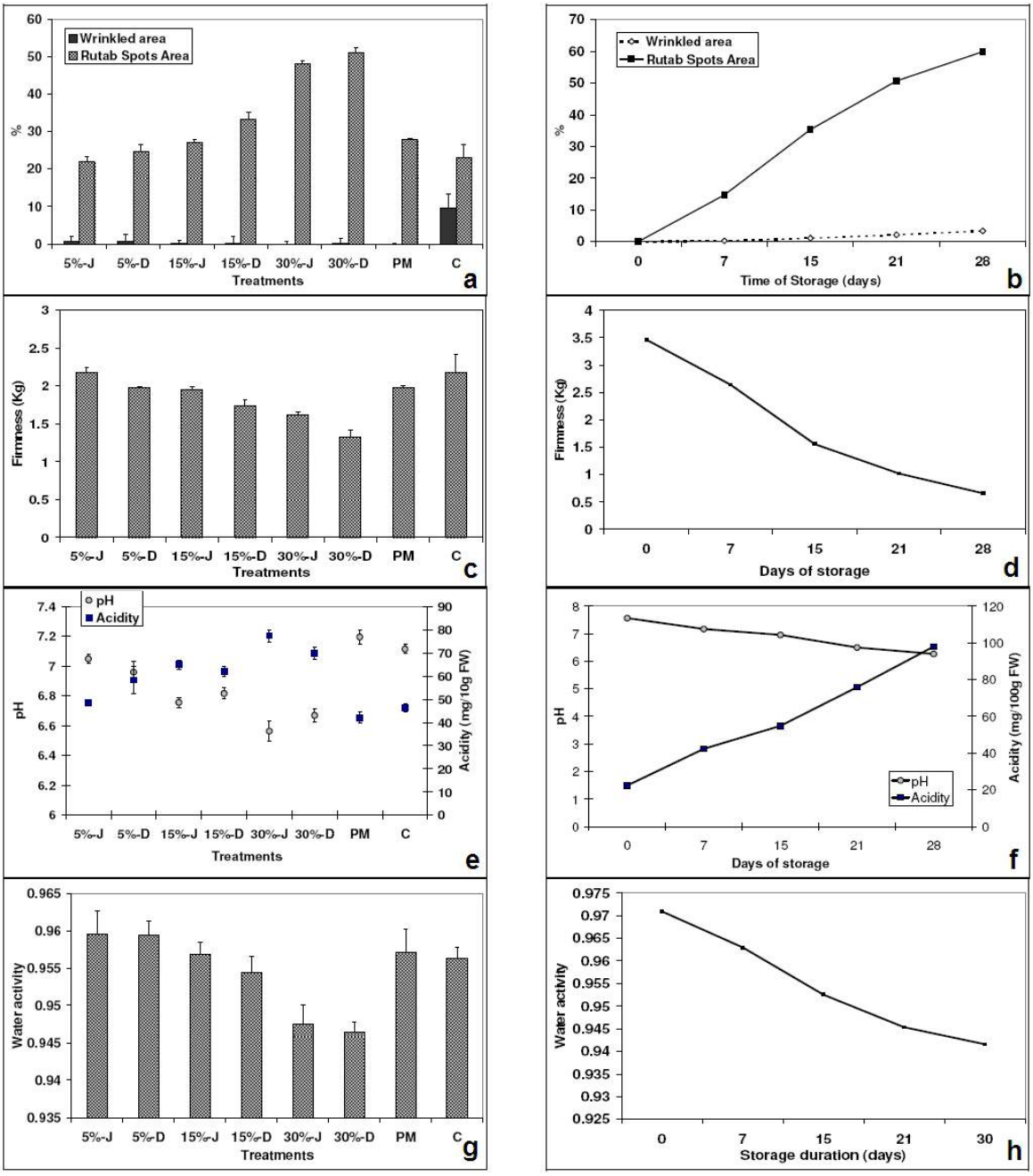

Fig. 2. The effect of applied treatments (left) and storage duration (right) on different quality attributes.

Evaluation of Using Midribs of Date Palm Fronds as a Raw Material for Wood-Cement Composite Panels Industry in Saudi Arabia

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Keywords: date palm, wood-cement panels, Portland cement, hydration test, compatibility

Abstract

This study was carried out in 2008 to investigate the suitability of date palm midribs (*Phoenix dactelifera* L.) as a lignocellulosic material for the production of wood-cement composite panels, in addition to enhancing their compatibility with cement using various pretreatments and chemical additives. Materials used for this study were midribs of fronds of different date palm cultivars available in Saudi Arabia and Portland cement (Type I) manufactured by Yammama Cement Company. To achieve this aim, hydration tests of net cement and date palm-cement mixture were carried out using a 2-L Dewar flask. The suitability of date palm for this industry was made according to inhibitory index (I) and compatibility factor (C_A) which were calculated from the hydration data. The results showed that under untreated condition, date palm particles are incompatible with cement and were classified as "unsuitable" for making wood-cement boards. Using of cold or hot water extraction for date palm materials resulted in an enhancement in their compatibility with cement. However, date palm can be reclassified as suitable under limited conditions for making wood-cement boards only by using hot water extraction. Addition of 3% $CaCl_2$ to untreated date palm particles resulted in it being reclassified to suitable under limited conditions (T_{max} value was 54.23°C and C_A value was 75.73%). These results suggested that date palm midribs can be used to produce wood-cement panels either after extraction by hot water or addition of 3% $CaCl_2$ as an accelerator to the date palm-cement mixture.

INTRODUCTION

Saudi Arabia does not posses adequate forest resources to meet their needs for fuel wood, industrial wood, and wood composition panels. However, it has relatively large quantities of other lignocellulosic materials available in the form of agricultural residues. The number of date palm trees exceeds 20 million (Ministry of Agriculture, 2001). A large quantity of this date palm population sheds a huge quantity of plant biomass annually from seasonal pruning as an essentially agricultural practice. According to El-Juhany (2001), 35 kg on average of palm residues is obtained per tree annually, about one million metric tons of date palm biomass is wasted annually from seasonal trimming of the palm tree population in Saudi Arabia. In developing countries most of these residues are burnt, however, in developed countries these residues are used to produce wood composites such as particle board and medium density fiber board.

The date palm (*Phoenix dactylifera* L.) is an important element of the flora in all the Arab countries. It plays a pivotal role in the economic, social and cultural life in the Arab region (El-Mously, 1997). Horticultural experts believe that the cultivation of the date palm tree started around 6000 BC with many uses of the trees. The wood and leaves provide timber and fabric for houses and fences. The leaves are used for making ropes, cord, baskets, crates and furniture. The base of the leaves and the fruit stalks are used as

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fuel.

In Egypt, the date palm midribs were successfully used in 'Mashrabia' handicrafts as a substitute for the imported beech wood, *Fagus sylvestris* (El-Mously, 1997), in the core layer of the block board as a substitute for the imported spruce wood (*Picea abies*) without sacrifice of the utilization properties of the product. Three layer particle boards were made of palm midrib as a substitute for casuarina wood, *Casuarina* spp. (Kandeel et al., 1988; El-Mousely, 1997). The Center for Development of Small Scale Industries and Local Technologies in Egypt has demonstrated the successful use of date palm fronds for the manufacturing of lumber-like products having physical and mechanical properties similar to those for imported wood (El-Mousely, 1997).

Wood-cement particle boards (WCP) refer to particles or fibers of wood or non-wood materials, which are used as reinforcement materials, bonded and held together by an inorganic binder such as ordinary Portland cement. Using varying methods and techniques, lignocellulosic materials from different countries have been screened, tested and classified according to their suitability for wood-cement panel production (Hofstrand et al., 1984; Shulka et al., 1984; Gnanaharan and Dhamodaran, 1985; Yasin and Qurashi, 1990; Semple et al., 2002; Olorunnisola, 2008). Based on these results wood-cement particle boards are generally made from wood species which have been tested and proven suitable.

Unlike resin-bonded particleboard, WCP show excellent sound insulation, high resistance to water and termites, and excellent long-term weatherability and outdoor conditions. These advantages lead to its wider potential applications for replacing traditional building materials and conventional wood composites as siding, roofing, wall, flooring parts, support tables, and noise absorbing partitions (Wei and Tomita, 2001). This interest in cement-bonded particle board (CBP) can be attributed to the high cost of resin and machinery necessary for production of resin-bonded boards, which are attractive to WCP (Ajayi and Badejo, 2005).

With all the advantages of WCP, however, the compatibility of some lignocellulosic materials with cement is limited, which may inhibit cement setting to some extent and limit WCP development (Moslemi and Lim, 1984; Gananaharan and Dhamodaran, 1985; Vaickelioniene and Vaickelionis, 2006). The water-soluble materials in wood have the greatest inhibitory effect (Sandermann and Kohler, 1964; Jai and Chen, 1977; Moslemi et al., 1983; Yasin and Qureshi, 1990; Nasser, 1996). Extraction of wood by hot or cold water and/or addition of chemical additives to wood-cement mixtures are extremely important in improving their compatibility (Lee and Short, 1989; Nasser, 1996).

The method developed by Sandermann and Kohler (1964) is commonly used to measure the compatibility of wood-cement mixture because it is reliable and very simple to be carried out. In this method, the heat of hydration produced during the setting of wood-cement mixture is measured. This compatibility was classified based on the extent to which they retarted cement hydration using compatibility factor (C_A) and inhibitory index (I) according to Sandermann and Kohler (1964), Hachmi and Moslemi (1989) and Okino et al. (2004).

The studies conducted in Saudi Arabia to produce wood-cement particle board and to evaluate the compatibility of agricultural residues for CBP manufacturing are limited. Therefore, the objective of the current study was to evaluate the suitability of the midribs of date palm fronds as a lignocellulosic raw material for manufacturing wood-cement products that have high economical value.

MATERIALS AND METHODS

Raw Material

The lignocellulosic material used for the current study was pruning residues of date palm (*Phoenix dactylifera* L.) which are midribs of fronds collected from the seasonal pruning process of date palm trees planted at the Agricultural Experimental

Station near Derab, 50 km south of Riyadh, Saudi Arabia. On the other hand and for comparison purpose, wastes from European redwood (*Pinus sylvestris* L.) were collected from wood stores in Riyadh city. These materials were air-dried then reduced to small pieces in order to facilitate grinding into meal. These were later put in a hammer mill using a prototype hammer mill and screened. Wood meal passing through a 20-mesh screen and retained on a 40-mesh screen was used for the hydration test, while those meals that pass through a 40-mesh screen and retained on a 60-mesh screen were used for chemical analysis.

Commercial ordinary Portland cement (Type I), meeting ASTM C150-84 specification (1984), and manufactured by Al-Yammama Cement Company was used as a binder.

Chemical Additives

Two chemical additives were used in this study, namely calcium chloride dehydrate ($CaCl_2.2H_2O$) and magnesium chloride ($MgCl_2$) at 1 and 3% of cement weight. Additives were dissolved in distilled water at least 15 min before adding to the untreated wood-cement mixture.

Cold and Hot Water Treatments

About 30 g of oven-dried wood meal (20-40 mesh) was soaked in cold distilled water (cold water treatment) or extracted in boiling distilled water (hot water treatment) for 48 or 3 h, respectively according to Moslemi et al. (1983) with some modification. The pH of the extracts was measured with an Accunet pH meter. Later, the extracted materials were used for the hydration test to determine their compatibility with cement.

Seven treatments were used in this study, namely wood+cement (untreated, UNTRT), wood extracted by cold water+cement (CWE), wood extracted by hot water+ cement (HWE), UNTRT+1% $CaCl_2$, UNTRT+3% $CaCl_2$, UNTRT+1% $MgCl_2$ and UNTRT+3% $MgCl_2$.

Chemical Analysis of Date Palm Midribs and European Redwood

The contents of cellulose, hemicellulose, total extractives and ash were determined for date palm midribs and European redwood according to the standard methods as follows:

1. Extractives Content Determination. The extractive content of wood was determined according to the ASTM, D1105-84 (1989). Samples of air-dry wood were chipped and ground to pass through 40-mesh screen and retained on a 60-mesh screen. Air-dried wood meal was extracted in a Soxhlet apparatus with ethanol-benzene mixture (in the ratio of 1:2 by volume, respectively) for 4 h, followed by extraction with 95% ethanol for 4 h, and finally extracted with hot distilled water for 4 h with changing water every 1 h. The percentage of extractives was calculated based on the oven-dry weight of wood samples.

2. Cellulose Content Determination. Cellulose content was determined by the treatment of extractive-free wood meal with nitric acid and sodium hydroxide: 1 g of extractive-free wood meal was treated with 20 ml of a solution of nitric acid 3% in flask and was boiled for 30 min. The solution was filtered in a crucible. The residue was treated with 25 ml of a solution of sodium hydroxide 3% and was boiled for 30 min. The residue was filtered, washed with warm water to neutral filtrate, dried and weighed. The cellulose content was calculated as percentage of residues based on oven dry wood meal weight (Nikitin, 1960).

3. Hemicelluloses Content Determination. Hemicellulose content of buttonwood samples was determined by the treatment of extractive-free wood meal (about 1.5 g) with 100 ml sulfuric acid (2%) and boiled for 1 h under a reflex condenser and filtrated in a crucible. After that the residue was washed with 500 ml of hot distilled water to free of acid, and contents were dried in an oven at 105±2°C, cooled in a desiccator and weighed (Rozmarin and Simionescu, 1973). Then, the hemicellulose content was calculated based on the oven-dry weight of the spacemen.

4. Ash Content Determination. Ash content of wood was determined according to the

Chemical Analysis and Testing Task Laboratory Analytical Procedure "NREL" (1994). Approximately 1 g of oven-dry sample was placed into the crucible. The sample in an uncovered crucible was heated gradually, then ignited at 575±25°C in muffle furnace for a minimum of 3 h, or until all the carbon is eliminated. Ash content was calculated as a percentage of residues based on the oven-dry wood meal weight.

Hydration Procedure

The hydration of net cement and wood-cement mixtures was carried out according to Hofstrand et al. (1984). 200 g of cement and 15 g of oven-dried milled wood (20-40 mesh) were mixed and kneaded with 90.5 ml of distilled water for about 2 min. The mixture was then placed into a 2-L Dewar flask. The thermocouple wire (Type T) was connected to a data-logger where the temperature was measured at 15-min intervals for 24 h. The time and temperature readings were plotted to obtain the exothermic hydration curve. The hydration data were used to calculate compatibility factor, C_A, (Hachmi et al., 1990), and inhibitory index, I, (Hofstrand et al., 1984). The 24-h limit was chosen for practical reasons in order to limit the hydration test duration (Okino et al., 2004).

The used experimental design was the split-plot design (Steel and Torrie, 1989). The main plot was lignocellulosic materials, while treatments (pretreatments and chemical additives) were used as sub-plot, with three replicates. The results were analyzed using the Statistical Analyses System (SAS, 1990). Least significant differences at 95% level of confidence ($LSD_{0.05}$) were used to detect the differences between means.

RESULTS AND DISCUSSION

The obtained results (T_{max} and t_{max}) and calculated (C_A and I) from the hydration test of date palm-cement and European redwood-cement mixtures are presented in Table 1. Since the main objective of this study was to evaluate the suitability of date palm midrib fronds as a raw material for manufacturing wood-cement particle board, in addition to improve their compatibility with cement, the obtained results will be discussed as follows:

Suitability of the Untreated Date Palm Midribs for WCP Industry

The statistical analysis of the current data revealed that all various hydration parameters of wood-cement mixtures differed between date palm and European redwood. Figure 1 shows the exothermic hydration curves of the untreated lignocellulosic materials with cement. It can be seen from this figure that each material reacted differently when mixed with cement. The extents to suppression temperature and t_{max} took place were measured for the retarding effect of the wood and non-woody lignocellulosic materials on cement setting. Wood which caused greater temperature depression is likely to be less suitable for WCP manufacture (Mohamed, 2004).

Comparing with net cement, the untreated lignocellulosic materials used in this study depressed the temperature rise and increased the time to reach maximum temperature during the setting process of cement (Table 1 and Fig. 1). European redwood (*Pinus sylvestris*) gave the higher T_{max} (52.50°C) and the lower t_{max} (9.93 h), while the lower T_{max} (38.17°C) and the higher t_{max} (24 h) were obtained for date palm midribs. According to these values, the former gave the higher C_A value (78.49%) and the lower I value (13.24%), while the latter gave the lower C_A and the higher I values (29.54 and 119.67%, respectively). The value of the inhibitory index of untreated date palm was very high (119.67%) due to long t_{max} and low T_{max} of hydration.

Accordingly and based on the obtained results as well as the classifications of Sandermann and Kohler (1964) and Hofstrand et al. (1984) the midrib of date palm fronds are extremely inhibitory and can be classified as "unsuitable" and require special pretreatments to reduce their inhibitory characteristics with cement. Whereas European redwood could be grouped as least inhibitory and was classified as "suitable under limited conditions" for making WCP panels.

The differences in the compatibility between date palm and European redwood under untreated conditions can be attributed to the differences in cold and hot water soluble substances of wood, which is determined as solubility and the pH of their extracts as well as to the differences in the type and quantity of hemicelluloses. It can be said that *Pinus sylvestris* as softwood species with the xylans in its polyoses are different from the date palm as monocotyledons type of polyoses in its chemical structure. The obtained results for solubility and pH of the extracts (Table 2) are consistent with the hydration characteristics results of the untreated materials. Date palm particles contain the higher hot and cold water substances and the lower pH values, whereas European redwood reacted differently (Table 2). Gnanaharan and Dhamodaran (1985) reported that species with low acidic extract along with low cold water solubility will be suitable for wood-cement-wool board manufacture. These results are in agreement with the results of research carried out in other parts of the world (Jai and Chen, 1977; Zhengatian and Moslemi, 1989; Mohamed, 2004).

Effect of Some Treatments on the Compatibility of the Date Palm with Cement

The results of the analysis of variance revealed that the treatments used in the present investigation affected significantly all hydration parameters of wood-cement mixtures. In addition, the interaction of kind × treatments is significant. This means that under different treatments used, each lignocellulosic material reacted differently when mixed with cement. Figure 2 shows the effect of different treatments on hydration characteristics of the date palm and European redwood materials.

For each lignocellulosic material there was a certain combination among the seven treatments used in this study that produced the best result. Using of cold or hot water extraction for the two materials used resulted in an enhancement in their compatibility with cement depending on the types of materials under consideration. This enhancement can be noted by changes in the hydration curves (Fig. 2), increase in T_{max} and C_A values, and decrease in t_{max} and I values of wood-cement mixtures (Table 3 and Fig. 2). There were very large variations in improvements which were obtained using cold or hot water extraction among the date palm and European redwood. Generally, enhancement obtained by hot water extraction was higher than in cold water extraction.

1. Effect of Pretreatments on the Compatibility. Under cold or hot water extraction, and based on the classification of Sandermann and Kohler (1964) and Okino et al. (2004, 2007), date palm particles were suitable under limited conditions only if they were treated with hot water since T_{max} is between 50 and 60°C (50.25°C) and C_A value is increased to reach 68.73%. No enhancement was obtained after extracted date palm particles by cold water. On the other hand, European redwood was reclassified from suitable under limited conditions to suitable for making wood-cement board by using either cold or hot water extraction (Table 3 and Fig. 3). This improvement can be attributed to removal of sugars and other water-soluble substances from woody and non woody materials used, especially for date palm, which appear to be highly inhibitory to setting of cement in their natural state. These results are conformed in this study by the addition of cold or hot water extracts of these materials to cement. The inhibitory effect of these extracts on cement setting can be noted by the increase in t_{max} and I values, and decrease in T_{max} (Fig. 3), ΔT, and C_A values.

2. Effect of Chemical Additives on the Compatibility. With regard to the addition of either $CaCl_2$ or $MgCl_2$ (1 or 3% based on cement weight) to the mixture of cement and untreated date palm midribs and European redwood, it is obvious from Table 4 and Figures 2 and 4 that each material reacted differently with cement when chemical additives were added to the mixtures. The highest T_{max} values were obtained for untreated European redwood-cement mixture after adding either 3% $CaCl_2$ or 3% $MgCl_2$ (69.73 and 69.53°C, respectively). Based on the classification of Sandermann and Kohler (1964) and Okino et al. (2004), date palm midribs were reclassified from unsuitable to suitable under limited conditions only if $CaCl_2$ was added, whereas European redwood was reclassified from suitable under limited conditions to suitable for making WCP by addition of either

$CaCl_2$ or $MgCl_2$ to untreated wood-cement mixtures. No improvements were achieved by adding 1% $CaCl_2$ or $MgCl_2$ (1 or 3% by cement weight).

Although addition of chemical additives to wood-cement mixtures in the current study improved the compatibility with cement, these additives did not appear to have neutralized the detrimental effect of high inhibitory date palm midribs on exothermic reactions of cement. This statement is in agreement with Moslemi et al. (1983) and Mohamed (2004) who found that the addition of 3% $CaCl_2$ to the mixture slightly improved the maximum hydration temperature for the untreated cotton stalks and bagasse particles by 17.6 and 18.49%, respectively but in contradiction with Nasr (2002) who reported that among different types of chemical additives ($CaCl_2$, $FeCl_3$, $MgCl_2$, and NaOH), the addition of 3% $CaCl_2$ to untreated cotton stalks-cement mixture gave the lowest decrease in I values.

When chemical additives act as accelerators in the case of European redwood, the improvements in various hydration parameters obtained in the current study can be attributed to speed up the rate of hydration of plain cement without reacting with the wood substances when combined with low inhibitory materials or to provide a more suitable pH for setting the wood-cement mixtures (Moslemi et al., 1983). These results are in agreement with those of Zhengtian and Moslemi (1989), Nasser (1996), Mohamed (2004) and Olorunnisola (2007).

There is a certain combination among the seven treatments used in this study that produced the best result for each material used. In the case of European redwood, all treatments used in this study gave almost high improvements, but addition of either 3% $CaCl_2$ or 3% $MgCl_2$ to the untreated wood-cement mixtures gave the best results, however, out of the seven treatments used, the addition of 3% $CaCl_2$ to the untreated wood-cement mixtures showed the best results for date palm. Under this treatment, only date palm can be used for making wood-cement particle board. Finally, it can be said that date palm midrib front particles were suitable to produce wood-cement particle board only if particles were treated either with hot water extraction or with added 3% $CaCl_2$ to the mixture.

CONCLUSIONS

According to the obtained results from the current study, the following conclusions may be drawn:

- Date palm midrib fronds are extremely inhibitory and can be classified as “unsuitable” and require special treatments to reduce their inhibitory characteristics with cement, however, European redwood was least inhibitory and classified as “suitable under limited conditions” for making WCP.
- The best treatments which proved effective for enhancing the compatibility of date palm midribs were either extraction with hot water or addition of 3% $CaCl_2$ as accelerator but we suggest the addition of 3% $CaCl_2$ to treated wood by hot water extraction which gave the best result.

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Tables

Table 1. Hydration data for the mixture of untreated date palm-cement mixture in comparison with European redwood-cement mixture and net cement.

Mixture	T_{max} (°C)	t_{max} (h)	ΔT (°C)	R (°C/h)	C_A (%)	I (%)
Date palm-cement	38.17^B	24.00^A	13.90^B	0.58^B	29.54^B	119.67^A
European redwood-cement	52.50^A	9.93^B	27.80^A	2.80^A	78.49^A	13.24^A
Net cement	83.60	6.28	59.20	9.43	100.00	0.00

Means with the same letters in the same column are not significantly differences at 5% level of probability according to LSD test.

T_{max}: maximum temperature.
t_{max}: time to reach T_{max}.
ΔT: rise in temperature above the ambient.
R: hydration rate.
C_A: compatibility factor.
I: inhibitory index.

Table 2. Chemical analysis of date palm midribs and European redwood used for wood-cement mixtures.

lignocellulosic materials	% of total material weight				Hot water		Cold water	
	Total extractives	Cellulose	Hemi-cellulose	Ash	Solubility (%)	pH	Solubility (%)	pH
Date palm	15.45^{A}	46.41^{B}	25.89^{A}	7.91^{A}	21.65^{A}	4.85^{B}	15.28^{A}	4.33^{B}
European redwood	6.65^{B}	53.47^{A}	16.99^{B}	0.30^{B}	6.03^{B}	5.70^{A}	3.13^{B}	5.87^{A}

Means with the same letters in the same column are not significantly differences at 5% level of probability according to LSD test.

Table 3. Maximum temperature (T_{max}), time to reach maximum hydration temperature (t_{max}), inhibitory index (I) and compatibility factor (C_A) for the mixtures of date palm-cement and European redwood-cement as affected by some treatments.

Species	Treatments: Wood	Treatments: Additives	T_{max} (^oC)	t_{max} (h)	I (%)	C_A (%)	Suitability*
	Untreated	None	38.17	24.00	119.67	27.85	Unsuitable
	CWE	None	41.10	13.57	54.30	27.75	Unsuitable
	HWE	None	50.25	11.43	28.78	68.73	SU
Date palm	Untreated	1% $CaCl_2$	39.83	24.00	77.79	30.48	Unsuitable
	Untreated	3% $CaCl_2$	54.23	11.89	28.28	75.83	SU
	Untreated	1% $MgCl_2$	42.27	24.00	66.95	41.45	Unsuitable
	Untreated	3% $MgCl_2$	40.93	24.00	75.82	28.29	Unsuitable
	Untreated	None	52.50	9.93	13.24	78.49	SU
	CWE	None	59.83	7.99	4.34	81.74	Suitable
European	HWE	None	62.67	8.23	4.41	86.44	Suitable
Redwood	Untreated	1% $CaCl_2$	63.67	5.04	-1.02	82.01	Suitable
	Untreated	3% $CaCl_2$	69.73	4.58	-0.56	90.52	Suitable
	Untreated	1% $MgCl_2$	55.37	6.23	-0.77	89.96	Suitable
	Untreated	3% $MgCl_2$	69.53	4.98	-0.39	92.94	Suitable
LSD 0.05 for species* treatments			2.13	3.64	11.88	5.93	
Net cement			83.6	6.28	0.00	100	

Each value represents the mean of three replications.
CWE: Wood extracted by cold water. HWE: Wood extracted by hot water.
SU: Suitable under limited conditions. * According to Sandermann and Kohler (1964).
For neat Portland cement: T_{max} (83.6 ^oC), t_{max} (6.28 h), I (0), and C_A (100%).
(-) Negative I values due to lower t_{max} of neat cement than t_{max} of wood-cement.

Figures

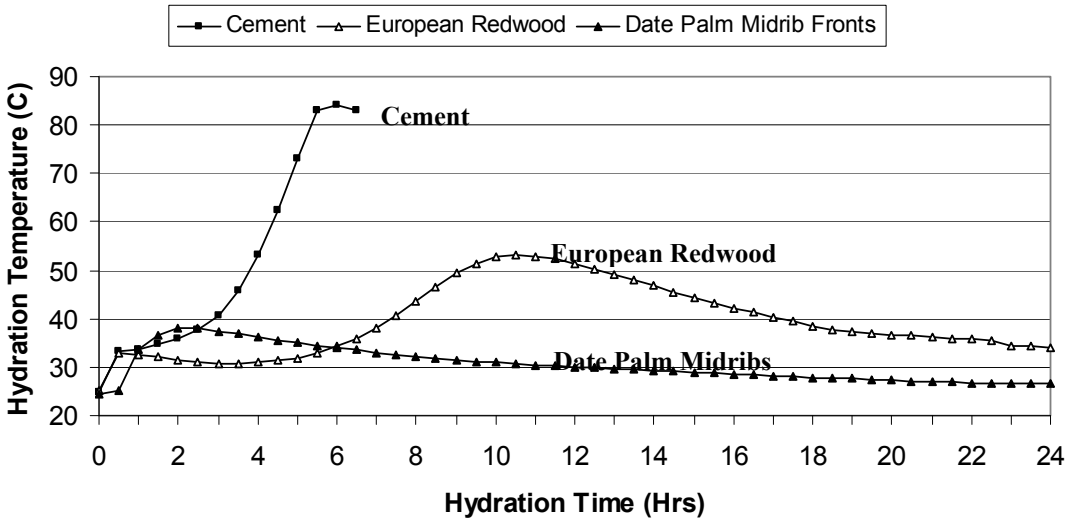

Fig. 1. Exothermic hydration curves of untreated date palm-cement and European redwood-cement mixtures without chemical additives comparing with net cement.

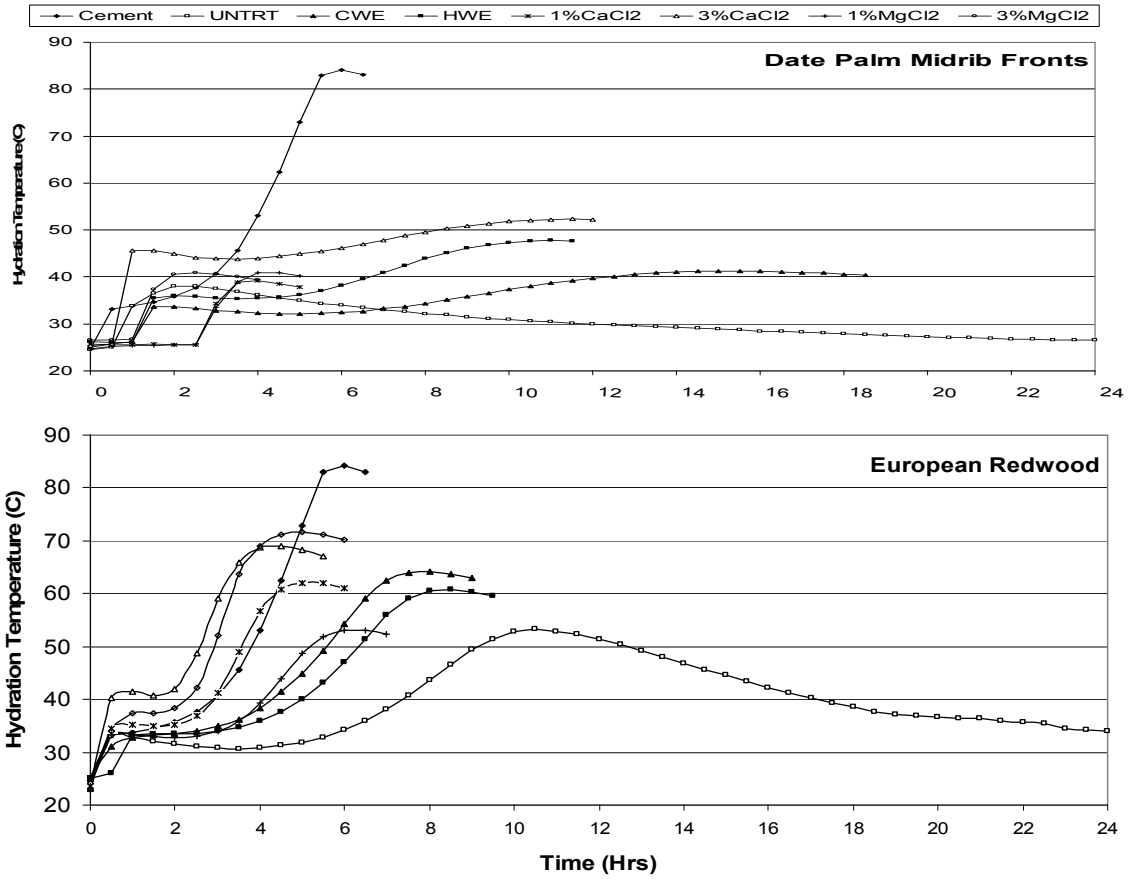

Fig. 2. Exothermic hydration curves of the mixtures of midribs date palm fronds and European redwood with cement under different treatments as compared to net cement. For legend see section of materials and methods.

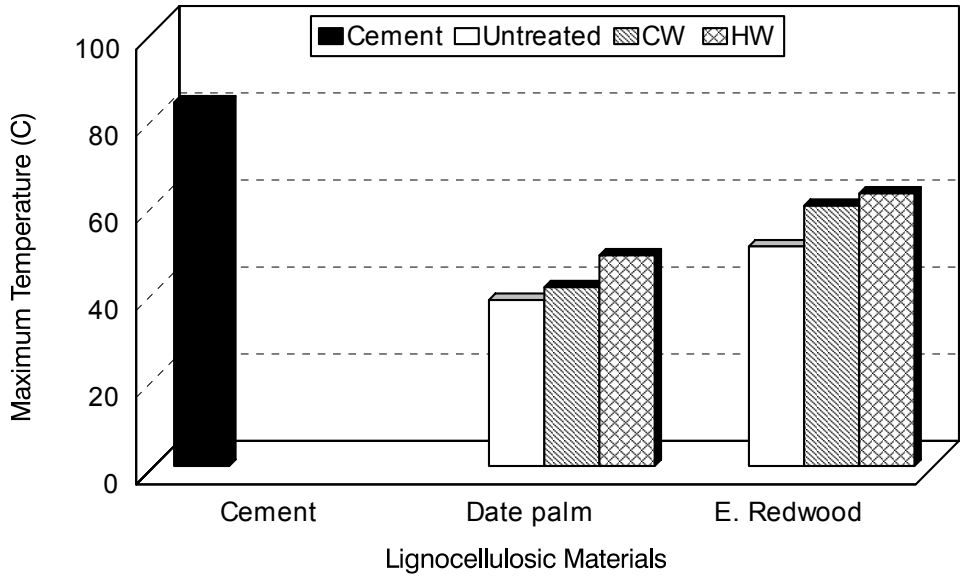

Fig. 3. Effects of cold water extraction (CW) and hot water extraction (HW) of date palm and European redwood on the maximum hydration temperature of wood-cement mixtures with comparing to cement and untreated wood-cement mixture (untreated).

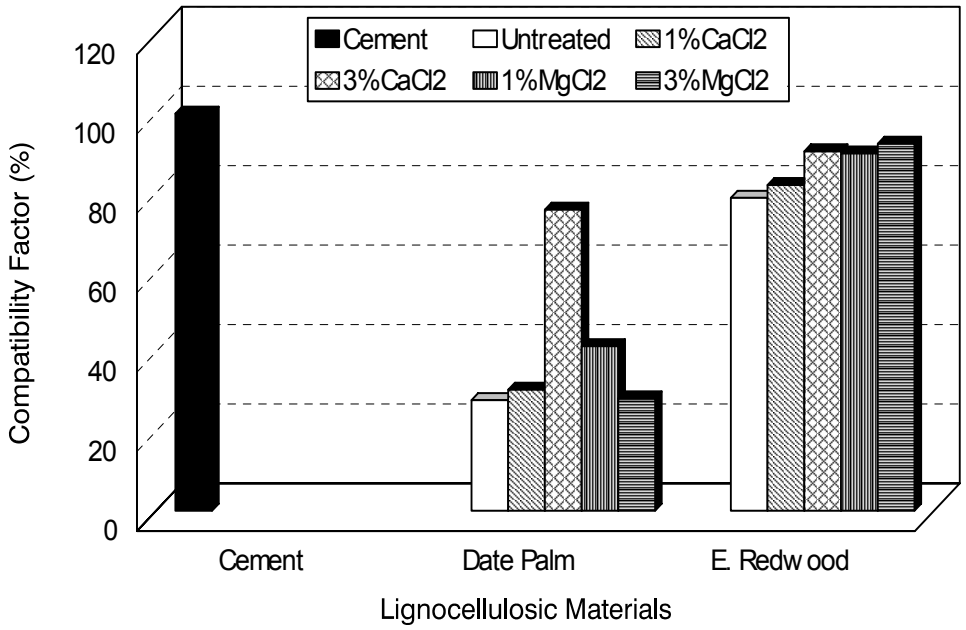

Fig. 4. Effects of chemical additives ($CaCl_2$ and $MgCl_2$) on the compatibility factor of wood-cement mixtures as compared to cement and untreated wood-cement mixture (untreated).

The Consumption Pattern of Dates and Its Related Food Habits among UAE Citizens in Al-Ain City, UAE: a Pilot Study

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Keywords: dates, consumption pattern, food habits, nutrition, United Arab Emirates

Abstract

Objectives: (1) to measure the level of general knowledge of UAE citizens about palm trees, dates and dates products, (2) to identify the consumption pattern of dates, and (3) to highlight the food habits related to dates consumption in Al-Ain city, UAE.

Subjects and methods: 209 UAE citizens aged 15-65 years were recruited by convenient, time sampling technique during January-February 2006 in Al-Ain City. A pre-tested questionnaire consisting of 3 parts: knowledge, consumption pattern and habits, was used to collect the data through face to face interview.

Results: the results of the knowledge part show very low scores (37/100) with significant differences according to gender in favor of males, while no differences were detected as per the subject's age. The average number of dates daily consumed was 8 dates with significant differences according to gender (males: 12, females: 7). A statistical proportional increase in the daily dates consumption was detected as the subject's age increases. Results showed that the percent of the subjects who ate dates daily was 63%, weekly: 23%, monthly: 10% and who never ate dates was 5%. The dates were consumed frequently in the afternoon time (41%) and in the evening (18.2%). The favorite dates were: 'Khlas' (53.1%), 'Khnaizi' (9.6%) and 'BuM'aan' (6.7%). About 21.3% of the sample did not know the name of the dates they are eating. About 47% of the children do like eat dates.

Conclusions and recommendations: this pilot study is one of the little studies highlighting the pattern of date consumption in UAE. The knowledge about dates can be described as a very weak level. More efforts should be spent to encourage younger people to eat dates. Adopting programs aiming at increasing daily consumption of dates is highly recommended.

INTRODUCTION

Date fruits are considered one of the most famous fruits in the Arab Gulf Area. The weather of this area that is characterized by high temperature and humidity in addition to scarcity of water helped people to cultivate the palm tree in their desert lands which provided them with a very nutritious food; the fruits of the palm dates are considered as a very vital and important components of the Arabian Gulf meal (Kaaka, 2003). As compared to other GCC, the United Arab Emirates is classified in the first rank regarding the number of cultivated palm trees, where about 40 millions of palm trees are cultivated in UAE, followed by Saudi Arabia (18 millions) and followed by Oman (8 millions) (Ibrahim, 2004). UAE has the fourth rank in dates production worldwide, producing about 755,000 tons, according to the Food and Agriculture Organization estimates in 2007 (FAO, 2007).

Although there are enough data about the number of palm trees and dates production, still very little information is available regarding the consumption of the palm dates in the UAE. Evidence of the presence as well as early consumption of dates in UAE is documented (Beech and Shepherd, 2001; Ishida et al., 2003; Mery and Tengberg, 2009). It is reported that dates and camel milk were the main sources of food in the UAE

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before the era of oil production. However, many dramatic changes in all life aspects, including the life style and consumption patterns, occurred due to the oil revolution, where there was a noticeable shift or transition state towards the consumption of new foods due to the improvements of food processing and new products development while reducing the consumption of traditional foods. Such shifting negatively affects the nutritional and health status of the individual, especially young children (Chen, 1999; Cruz, 2000). Changing of food habits to healthy ones is a very difficult process and takes a lot of time since it is affected with a complex of factors as physiological, socio-demographic, ethnicity and knowledge and attitudes related to diet and health (Terry et al., 1991; Slack et al., 1996). Chocolates, chips, candies, sweets, and other foods are unfortunately replacing the healthy food. This replacement may occur due to the role of media or lack of knowledge in addition to the little intervention of the family and its limited role in educating themselves and their children (Crockett and Sims, 1995; Birch and Fisher, 1998; Baranowski et al., 1999; Birch, 1999). Childhood is an important time to formulate healthy food habits among children, which then can persist into adulthood (Kelder et al., 1994; Lien et al., 2001), so that children prefer foods to which they have been previously exposed (Birch, 1999). Many research mentioned the important role of dates in nutrition and health. Miller et al. (2003) found that date, either alone or with yogurt, has a low glycemic index, and may be of benefit in glycaemic and lipid control of diabetic patients.

Hashim (2001) has mentioned and documented that many food products have been developed using dates, dates paste and dates dips as jam, jelly, date bars, candy, date juice, date juice milk drink, ice cream and tamr-eddin, substitute for Qumerdeen.

Very little studies reported the consumption pattern of dates among Emeratis. Examples of these studies include the work done by Ismael et al. (2006) and Musaiger and Abuirmeileh (1998). This rapid survey was conducted (1) to measure the level of general knowledge of UAE citizens about palm trees, dates and dates products, (2) to identify the consumption pattern of dates, and (3) to highlight the food habits related to dates consumption in Al-Ain city, UAE.

METHODOLOGY

209 UAE citizens aged 15-65 years visiting the primary health care centers were recruited by convenient, time sampling technique during January-February 2006 in Al-Ain City. A pre-tested questionnaire consisting from 3 parts: knowledge, consumption pattern and habits, was used to collect the data through face to face interview. The level of knowledge was assessed by a scoring system consisted of a maximum score of 100, which was expressed as values of mean±standard deviation. Other results were presented as percentages. Data were entered by Epi Info software and analyzed by SPSS 13. Independent student t-test and one-way analysis of variance were used to compare the means. Differences were considered statistically significant when p-value <0.05.

RESULTS AND DISCUSSION

Age and Sex Distribution of the Studied Sample

Table 1 shows some characteristics of the recruited sample. About 55% of the participants are aged between 21-30 years, followed by the age group 31-40 years (22.3%). About 72% of them are females. This predominance of female may result from the fact that most of men were at work during the morning time during which we collected our data.

Participants' Knowledge about Palm Trees and Dates in UAE

1. Scores of Knowledge Level Test. A very weak knowledge level was observed, with an average score of 37 out of 100 (Fig. 1). The males' knowledge level was significantly higher than that of females (39.6 vs. 34.3, respectively). Although there was no statistical association found between the age of participants, the highest knowledge was for the age

group of 50 years and above. We did not investigate the educational level of the participants as it was a rapid survey. A need for more deep and detailed questionnaire for measuring the level of participants is highly recommended.

2. Some Fields of the Knowledge Test. Results in Table 2 showed some fields of the knowledge test. About 39% of participants correctly answered the question about number of palm trees in UAE, while half of them correctly answered the question about the rank of UAE as dates producer worldwide. None of the participants knew the number of types of produced dates in UAE. The most famous types of dates as reported by the participants were 'Ekhlas', 'Khnaizi', 'Lou Lou' and 'BuM'aan'. Unfortunately, the participants' knowledge about the traditional food that is prepared with dates was very disappointing. It seemed that urbanization and modernization affect negatively the tight bonding of the participants with their culture, leading to the transitional shift towards the new food products as outcomes of food processing technology, especially in the three previous decades when UAE as well as other GCC countries became an open market for the huge number of food products from all parts of the world.

3. Food Habits Related to Dates Consumption. As is shown in Table 3, about 63.1% of the participants ate dates on daily basis, 23.1% on weekly basis, and 8.9% on monthly basis while about 5% never eat dates. Although about two thirds of them eat dates daily, still this percent is very low in a country ranked the fourth in date production on the world level. About 41% of the participants preferred eating dates in the afternoon time, while about 22% of them had no specific time for that. Most of the consumed dates are 'Ekhlas', as reported by about 53% of the respondents. As per the local vs. imported dates, the majority of the participants (70.2%) preferred the locally produced dates rather than the imported ones that are consumed by about 27% of UAE citizens. This latter percentage should be reduced in favor of local dates to encourage the local products that support the economy, taking into consideration that the UAE production of dates reached to about 757,000 tons in 2000-2003 (UAEAIC, 2003) and also UAE is considered the 6th dates exporting country in the world (FAO, 2004). It is reported that about half of the respondents preferred eating the natural, unprocessed dates and about 80% of them preferred eating both rutab and tamr dates. In non-tabulated results, it was found that about half of the respondents ate dates between the main meals, 74% of them eating the dates with the family, and they preferred eating dates with coffee (60%). It is found also that 86.7% of children preferred eating regular dates and the ones filled with nuts. About 36% of the children preferred eating the regular dates only. This trend is also found among the parents.

4. Amount of Daily Consumed Dates. The results of this pilot study revealed that a UAE citizen eats on average about 8 dates daily (about 80 g) (Fig. 2). These results almost resemble the ones found by Ismail et al. (2006). They found the average daily consumption of dates was 10 dates per day per person (about 114 g daily). Our findings showed that males consume dates more than females (12, 7 dates/day; respectively) with highly significant differences ($p=0.0001$). Furthermore, it is found that the daily consumption of date increased significantly with age (Fig. 3). The highest consumption was reported by the age group more than 50 years of age (17 dates/day) which then reduced to 11 dates/day for the age group 41-50 years. The average consumption of the younger age groups was about 7-8 dates/day. It is expected that older ages consumed a higher amount of dates than the younger ones. This trend was also notices by Ismail et al. (2006). The nutritional value for date is documented in many articles, explaining its health benefits for all ages. Encouraging children for consuming dates will improve their nutritional and health status as well as tighten their bonds to their culture as date palm is widely cultivated in UAE.

CONCLUSIONS AND RECOMMENDATIONS

This pilot study is one of the recent studies highlighting the pattern of date consumption in UAE. The knowledge about dates can be described as a very weak level. Although the daily consumption of dates is satisfactory, more efforts should be spent to

encourage younger people to eat dates as their daily consumption is significantly lower than older people. Adopting programs aimed at increasing the daily consumption of dates as traditional food is highly important. In depth research in this field is also highly recommended.

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Tables

Table 1. Some characteristics of the sample.

Characteristics		No.	%
Age	>= 20 yrs	26	12.6
	21-30 yrs	113	54.9
	31-40 yrs	46	22.3
	>= 41 yrs	21	10.2
Sex	Male	58	27.8
	Female	151	72.2

Table 2. Some knowledge fields about palm trees and dates in UAE.

Question		No. and % of correct answers
Number of palm trees in UAE (40 Millions)		82 (39.2)
Rank of UAE as dates producer worldwide (Rank 4)		107 (51.4)
Number of the types of dates produced in UAE (about 120 types)		0 (0.0)
Non food benefits of palm tree	Trunk	56 (26.8)
	Fronds (leaves)	145 (69.4)
	Environmental	25 (12.0)
	Pits	15 (7.2)
The 4 most famous dates produced in UAE (multiple responses)	Ekhlas	158 (75.6)
	Khnaizi	108 (52.1)
	Lou Lou	85 (40.7)
	BuM'aan	81 (38.7)
Food products of dates	Dibs	86 (41.1)
	Dates sweets	48 (23.0)
	Dates biscuits	46 (22.0)
	Maa'moul	22 (10.9)
	Dates cake	16 (7.6)
Traditional foods prepared by dates	Bitheeth	56 (31.1)
	Sweeten bread	32 (15.3)
	Aseedat dibs	26 (12.4)
	Mamrousa	11 (5.3)
	Loquimat B dibs	10 (4.9)
Number of dates factories in UAE	One factory	70 (33.5)
	Two factories	30 (14.4)
	Three factories	15 (7.2)
	Four factories	14 (6.7)
	More than four factories	16 (7.7)

Table 3. Food habits related to dates consumption in UAE.

Food habit		No.	%
Dates consumption pattern	Daily	128	63.1
	Weekly	47	23.1
	Monthly	18	8.9
	Never eat	10	4.9
Time of eating dates	Morning	20	10.1
	Between morning and afternoon	18	9.1
	Afternoon	81	40.9
	Evening	36	18.2
	No specific time	43	21.7
Type of consumed dates	Ekhlas	111	53.1
	Khnaizi	20	9.6
	BuM'aan	14	6.7
	Lou Lou	11	5.3
	Fard	6	2.9
	Do not know the type	45	21.5
Preference of consuming local vs. imported dates	Local	139	70.2
	Imported	5	2.5
	Both	54	27.3
Preference of consuming processed vs. non processed dates	Natural, non processed dates	94	48.0
	Processed	15	7.6
	Both	87	44.4
Preference of consuming dates vs. rutab	Dates	22	11.1
	Rutab	19	9.6
	Both	157	79.3

Figures

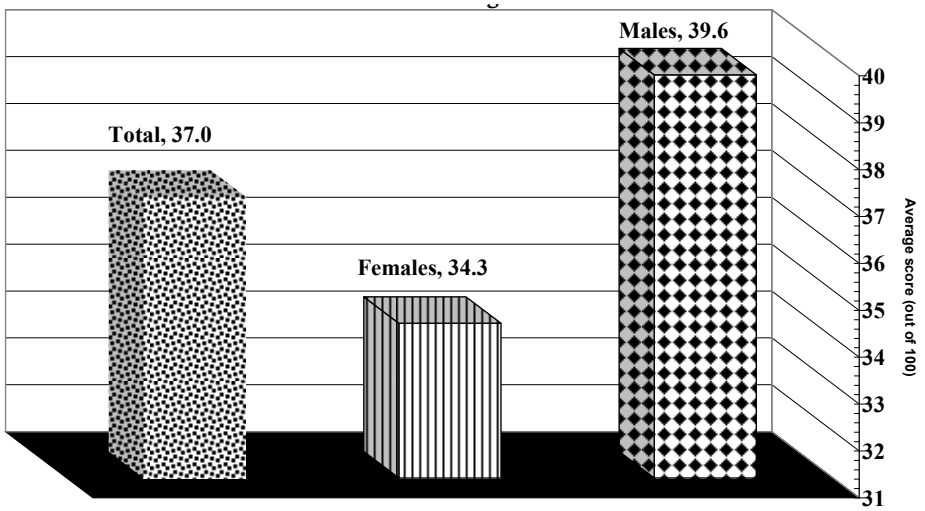

Fig. 1. Results of participants' knowledge about palm trees and dates in UAE according to sex (significant differences between males and females, t=2.05, p=0.042).

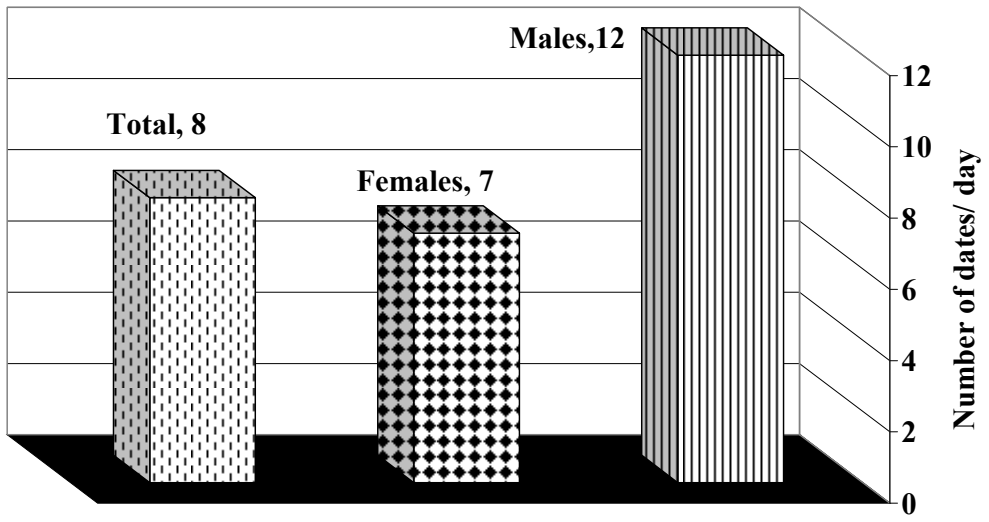

Fig. 2. Number of daily consumed dates according to sex (t=3.575, p=0.001).

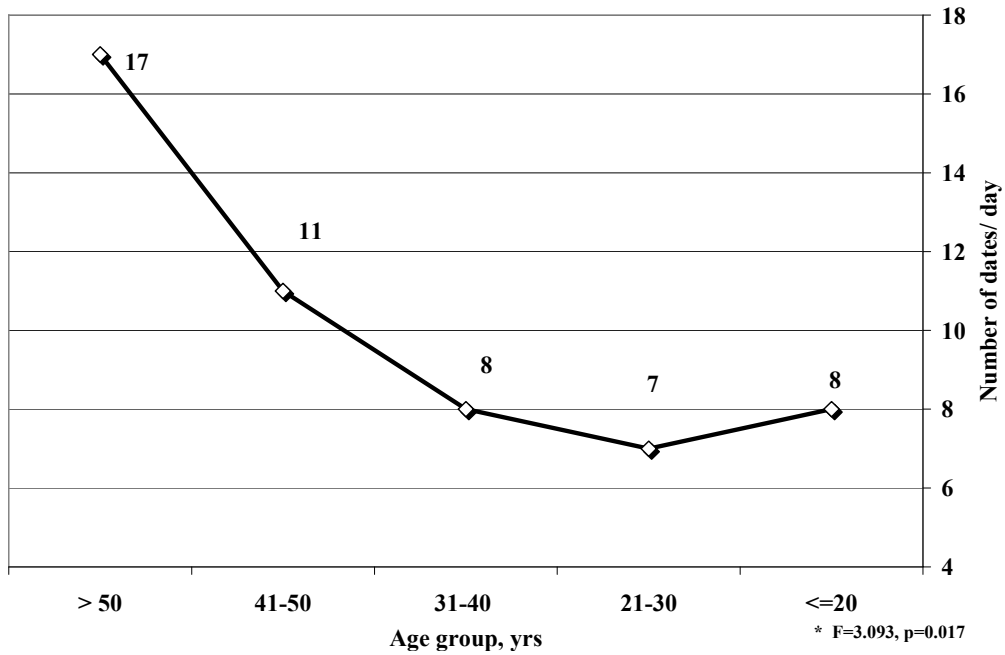

Fig. 3. Average number of daily consumed dates by age group.

Effect of Bunch Bagging on Productivity, Ripening Speed and Postharvest Fruit Quality of 'Zaghloul' Dates

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Keywords: ripening, bagging, quality, shelf life, date palm

Abstract

A field study was carried out during the 2007 and 2008 seasons on adult trees growing in sandy soil in order to obtain early fruit maturity and improve productivity and postharvest fruit quality. Spathes bagging was carried out at pollination time using transparent and blue polyethylene bags. Bags were removed after fruit set and when the fruit reached either to the hababouk (earliest stage of development), khimri or khalal stage (partially-ripe or biser). In general, the polyethylene bags caused a significant early fruit ripening, increased fruit weight, length, diameter and yield, while the percentage of cracked fruits at harvest time was decreased as compared to the control. As for the maturity, the transparent polyethylene bags and the removal of bags at khalal stage were more effective than the blue bags and removal of the bags at hababouk or at khimri stage whereas, the blue polyethylene bags and removing the bags at hababouk or khimri stage were the best for improving the fruits' quality at harvest time or during cold storage. In this respect, the shelf life and storability were higher in the blue polyethylene bags and removal of the bags at the hababouk or than the transparent polyethylene bags and removal of the bags at the khalal stage. The percentage of fruit drop was increased when the bags were removed at khalal khimri stage. Stalk length was clearly increased as a result of bagging and the increase was proportional to the duration of bagging.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the ancient domestic fruit trees in the Middle East countries and their fruits play an important role in the nutritious pattern of many people. According to FAO (2009), Egypt is considered the first country of the top ten date producers (1,130,000 tons). In Egypt many cultivars are grown in different regions according to the diversity of their climatic necessity, particularly average temperature and relative humidity that effect fruit growth and development. 'Zaghloul', 'Samany', 'Hallawy' and 'Hayany' seem to be the most early ripening cultivars of soft date grown in Egypt. 'Zaghloul' date is one of the most important commercial cultivars in Egypt and highly demanded on the Egyptian and Arab markets. Farmers are forced to early ripening as they always get the advantage of obtaining high economical revenues as they reach to the early ripening stage to increase the amount of fruits for export and before rain that causes top fruit cracking and acceleration of fruit decay. The top fruit cracking in 'Zaghloul' date palm is a physiological disorder and occurs very suddenly and rapidly at khalal stage. Many factors have been thought to have effects on this disorder such as fruit overloading and weather fluctuation especially high temperature and blowing hot winds could have influence on development of this disorder. Periods of high humidity

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immediately before the khalal stage, while the fruit is just beginning to fade a little in transition but still green, often cause minute superficial breaks, or checks in the skin and these vary in the different cultivars (Kassem et al., 1994). Dowson (1982) reported that when air humidity is high during maturation the skin of the date fruit shows several cuts or breaks with an edge-blackening (blacknose), the soft fruits fall to the ground and consequently lose their commercial value. 'Zaghloul' checking occurs mainly near the tip of the fruit and it is one of the most important factors that decrease fruit quality at harvest and during cold storage or marketability (Kassem et al., 1994). There is a trend of higher export to the Arab market. However, the exporting season starts early in Mid-September before the natural fruit maturity. Therefore to increase the exported amount of fruits some treatments should be done to cause early fruit maturity. Thus, many researchers have been attempting to accelerate ripening of dates by an ethylene-releasing compound, namely ethephon has been used to achieve these goals (Balaket, 1988; El-Hammady et al., 1992). The use of polyethylene bags to cover the spathes (bunch bagging) caused also early fruit ripening (Kassem et al., 1994; Awad, 2007). In this respect, the black and blue polyethylene bags were the most effective followed by white and paper bags (Awad, 2007). Furthermore, early bagging of polyethylene bags at pollination resulted in significant early fruits ripening, improved yield, enhanced fruit quality and make it harder to detect a developing fruit top cracking problem (Kassem et al., 1994). Bagging raised the temperature around the bunches and reduced the shooting to harvesting time, in addition, the microclimate of bunch could favourably be changed by bunch covering so that the bunch weight could be increased by 18-23% , thus it can be used also to improve the fruit appearance quality and to caused early fruit ripening (Chillet and Jannoyer, 1996). Wade et al. (1973) reported that in the protection of bunches from UV radiation, which otherwise causes necrotic scorching of the fruit peel, bunch bagging was also effective against sunburns and blemishes caused by wind-blown dust and birds or insects (Samson,1980; Anon., 1995).

The objectives of this study were to evaluate the effect of different bagging treatments on fruit development and ripening rate of 'Zaghloul' dates and increase its intensity in Edko region and to compare the performances of blue polyethylene bags with the conventionally used transparent polyethelene bags. These bags were removed after fruit set and when the fruit reached either to the hababouk, khimri or khalal stage (partially-ripe).

MATERIALS AND METHODS

Experiments were performed during the two successive seasons of 2007 and 2008 on 21 uniform 20-year-old 'Zaghloul' palm grown in sandy soil in a private orchard of Edko region, El-Behera Governorate near Alexandria. The palm trees were subjected to the same usual horticultural practices and were pollinated from the same male trees. The number of spathes per palm was adjusted to 10 by removing excess spathes and were bagged by transparent and blue polyethylene bags. The spathes were kept under bagging for one of the following stages: hababouk, khimri or khalal stage (partially-ripe).

Only 9-10 bunches of nearly equal size were left on each tree. The numbers of strands in every bunch were reduced to 60 and 65 by thinning out the excess from the bunch center.

The experiment consisted of 7 treatments on 21 trees by Complete Randomize Design with 3 replicates (each replicate=tree). The polyethylene covers are placed like sleeves over the bunches as soon as the pollination time is completed. They are tied at the top and left closed at the bottom. The schedule for bunch covering is usually every one to two weeks. Treatments arranged in a complete randomized design were as follows: T1) control; T2) transparent polyethylene bagging even at hababouk stage; T3) transparent polyethylene bagging even at khimri stage; T4) transparent polyethylene bagging even at khalal stage; T5) blue polyethylene bagging even at hababouk stage; T6) blue polyethylene bagging at khimri stage; T7) blue polyethylene bagging even at khalal stage.

In order to determine the effect of the different treatments on fruit physical and

chemical characteristics, a sample of ten strands were randomly collected from each bunch/replicate during both seasons at the commercial harvest date when the control fruits reached full maturity and red color. To study the effect of the different treatments on fruit storage ability and shelf life, a second fruit sample of 25 strands was randomly collected from each replicate when every treatment reached full maturity and red color and harvest date for each treatment in both seasons was recorded. Strands were kept at 0°C and 85-90% relative humidity for 30 days and the incidence percentages of fruit rutab, decay and weight loss were determined every 15 days during the cold storage to determinate the storage life.

Yield Components

The percentage of fruit set was determined according to El-Makhtoum (1981). Fruit drop percent was also calculated as the number of fruits that dropped in three months after fruit set and related to the total number of fruits setting. Seedless fruit percent and average of bunch weight were recorded at harvest time.

Fruits Physical Properties

The fruit physical properties were determined at harvest time; the average percentage of fruit top cracking (calculated as percentage of total fruits on each bunch), fruit and pulp weight (g) fruit width (diameter) and length (cm), shape index (fruit length/diameter) seed weight. Also, ground fruit color (assessment of color change) was estimated by giving ten degrees of red color stage, fruit red skin color in the 'Zaghloul' fruits was assessed visually and recorded on a scale from 0 (no color change) to 10 (complete change).

Fruits Chemical Properties

The fruits chemical properties were determined at harvest time; the percentage of fruit total soluble solids was measured by a hand refractometer, fruit acidity percent was determined by titration according to AOAC (1995).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) to detect treatment effect. Mean separation was performed by using least significant difference (LSD) at the $p \leq 0.05$ level. The data were analyzed using statistical analysis system (SAS, 1990).

RESULTS AND DISCUSSION

Yield Components

The data in Table 1 showed that all the bagging treatments caused an increase in the percentage of fruit set, fruit drop and yield as compared to the unbagged (control) during both seasons. Data also show that the all blue polyethylene bags treatment had a greater influence on fruit set, drop and yield than transparent polyethylene bags. No significant differences were observed between the transparent polyethylene treatments on the one hand and blue polyethylene treatments on the other hand for fruit set. In general, the transparent polyethylene bags and removal of the bags at khalal stage had the highest fruit drop and yield compared to the other transparent polyethylene bags treatment. Similarly, the blue polyethylene bags and removal of the bags at khalal stage had the highest fruit drop and yield compared to other blue polyethylene bags treatments. The highest fruit setting was observed with blue polyethylene bags treatments as compared to the transparent polyethylene bags treatments. Minimum fruit setting was reported in the control. Similar results were found with Kassem et al. (1994) who found that the bunch bagging treatments increased fruit set, drop and yield. Awad (2007) reported that the bunch bagging, especially with blue polyethylene bags, accumulated higher heat units than the control and the transparent polyethylene bags. Weerasinghe and Ruwanpathirana

(2002) found that the bunch covering increased bunch weight by 32%. They added that the bags' color did not affect the bunch weight or fruit weight. Reuther and Crawford (1946) reported that low temperature in the first half of the pollination period significantly decreased fruit set percent. This increase in fruit set might probably be due to the increase in temperature degree in the bagged spathes by about 5-15°F compared to unbagged ones and reduced the loss of pollens by the wind or the rain in the pollination period. Also Al-Baker (1972) and Hussein et al. (1979) agreed that bunch bagging increased the date yield. This increase might probably be due to the increase the temperature degree and the relative humidity which kept stigmas under bags fresh and crisp much longer than those on exposed flowers and prolonging the period of receptivity or by reducing the loss of pollens by wind or the rain and reducing the fruit drop percent.

Top Fruit Cracking

Data in Table 2 indicate that the percentage of top fruit cracking was decreased by transparent or blue polyethylene bagging treatments even at the khalal stage (T3 and T6) only during both seasons as compared to the other treatments. No significant differences were obtained among the other treatments. In this respect, the most effective treatment on decreasing the top fruit cracking was with the blue polyethylene bags followed by the transparent polyethylene bags especially when bagging remained even at the khalal stage. Izadi (2008) found that the bunch bagging decreased fading physiological disorder up to 70% and showed that climatic factors affected dates fading disorder by the following effectiveness sequence: weather relative humidity > wind speed and streamline > weather temperature. Also, estimation of fading disorder time occurrence can be done by wind streamline forecasting maps through five former days. It was concluded that climatic parameters could be the most important factor of fading disorder and there are three cultural control methods (intercropping, bunch covering, fruit thinning).

Fruit Physical Properties

Data in Table 2 show that the fruit weight, length and diameter were significantly increased by all bagging treatments when compared with the control during both seasons. The T2 ,T3 ,T5 and T6 bunch bagging treatments caused an increase in seed weight in the second season only. No significant differences were found between all the treatments. The most effective treatment on increasing fruit weight, length and diameter and seed weight was with the blue polyethylene bagging even at khalal stage. The red color increased with the transparent polyethylene bagging even at khalal stage and blue polyethelene bagging even at hababouk stage (T4), at khimri or at khalal stage in the first season and with transparent or blue polyethelene bagging even at khalal stage in the second season. Awad (2007) reported that the bunch bagging treatments, especially with black and blue polyethylene bags, accumulated higher heat units than the controls. Consequently, accumulated heat might induce higher heat units and the largest amount of heat units caused higher fruit growth rates under bagging treatments. Early bagging with polyethylene bags at pollination resulted in a significantly early enhanced fruit quality (Kassem et al., 1994). According to Chillet and Jannoyer (1996), the microclimate of the bunch could favorably be changed by bunch covering. Consequently, there is an increase in the bunch weight, thus it can be used also to improve the fruit appearance quality. Samson (1980) observed a temperature rise of 1.1-1.6°C surrounding the bunch and increasing the banana bunch weight by 1 kg. Al-Baker (1972) reported that spathes bagging for 30 days and for additional 15 days improved fruit shape and reduced fruit infertility by insects. However, Awad (2007) found that bunch bagging had no significant effect on the physical fruit characteristics such as fruit, flesh and seed weight, and fruit length and diameter.

Fruit Chemical Properties

Results in Table 2 indicate that fruit acidity did not show any definite trend as a result of any bunch bagging treatments. The fruit TSS content increased with the blue

polyethylene bagging even at khalal stage during both seasons and with the blue polyethelene bagging even at khimri stage in the second season only. However, it decreased with transparent polyethelene bagging even at khalal stage during both seasons (Table 2). Awad (2007) reported that the blue polyethylene bags decreased fruit TSS and acidity concentrations compared to the control. In the same time, he added that the white polyethylene bags decreased the fruit acidity.

HARVESTING DATES

The beginning of the harvest season in the non-covered bunch (control) was late in mid-October as compared with the bunch bagging treatments. Covering bunches with blue or transparent polyethelene bags even khimri or the khalal stage (bagging) can speed up the maturity which leads to ealier harvesting of the fruits. The results of this research showed that the ripening date of the covered bunches even khalal stage was faster than either khimri or hababouk stage (Table 3). Data also showed that the blue or transparent polyethylene bags caused a short spread of harvest. Awad (2007) reported that bunch bagging especially with black and blue polyethylene bags, accumulated higher heat units than the controls. Consequently, accumulated heat might induce higher respiration rates and the CO_2 accumulation within bags might lead to more acetaldehyde production and removal of astringency then hasten fruit filling and cause early fruit ripening.

STORABILITY

Data in Tables 4 and 5 indicate that bunch bagging with blue or transparent polyethylene bags even the khalal stage had the highest fruit weight loss and rutab percent after 15 or 30 days from cold storage as compared to the control, T1, T2, T4 and T5. No significant differences were obtained among the previously mentioned treatments during both seasons. Bagging with blue polyethelene bags even khalal stage and transparent polyethelene bags even at hababouk or khalal stage had the highest fruit decay and rutab percent, during both seasons as compared with the control. In the T1, T4 and T5 treatments no significant differences were obtained among the previously mentioned treatments after 15 days from cold storage. In the same time T3 and T6 had the highest fruit decay and rutab percent after 30 days as compared with the other treatments. From the results of our research it can be concluded that the bagging treatments even at khalal stage affected fruit storability compared to the unbagged (control) or bagging even at the hababouk and khimri stages.

CONCLUSIONS

It is recommended to cover the bunches directly at pollination with blue or transparent polyethylene bags even at hababouk or khimri stage in order to obtain earlier ripening as well as to improve their physical and chemical specifications by using blue polyethylene bags. Bagging with polyethylene bags even at khalal stage decreased fruit TSS and storability as compared to control or bagging even hababouk or khimri stage.

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Tables

Table 1. Effect of bagging treatments on fruit set, drop and yield during 2007 and 2008.

Treatment	2007			2008		
	Fruit set (%)	Fruit drop (%)	Yield (kg/palm)	Fruit set (%)	Fruit drop (%)	Yield (kg/palm)
T1	62_{c}	13.3_{e}	186_{f}	58_{c}	11.8_{e}	158_{d}
T2	71_{b}	17.7_{c}	208_{e}	68_{b}	21.6_{cd}	171_{bc}
T3	69_{b}	21.6_{b}	212_{de}	70_{b}	27.9_{ab}	183_{ab}
T4	73_{b}	28.8_{a}	238_{a}	68_{b}	30.6_{a}	186_{a}
T5	79_{a}	15.6_{d}	221_{c}	81_{a}	18.2_{d}	164_{cd}
T6	84_{a}	18.7_{c}	224_{bc}	85_{a}	23.4_{bc}	174_{abc}
T7	82_{a}	22.6_{b}	231_{ab}	83_{a}	26.6_{abc}	179_{ab}

Values within a row with the same letter are not significantly different ($p<0.05$).

Table 2. Effect of bagging treatments on top fruit cracking and fruit physical and chemical properties during 2007 and 2008.

Treatment	Top fruit cracking (%)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Seed weight (g)	Ground fruit color	Acidity (%)	TSS (%)
				2007				
T1	29.7_{a}	22.5_{e}	5.63_{e}	2.53_{d}	2.12_{a}	8.8_{b}	0.11_{a}	26.7_{bcd}
T2	27.9_{a}	24.4_{d}	5.98_{cd}	2.72_{c}	2.08_{a}	9.3_{ab}	0.17_{a}	25.9_{d}
T3	30.1_{a}	26.4_{c}	5.87_{d}	2.84_{b}	2.34_{a}	9.2_{ab}	0.21_{a}	27.6_{ab}
T4	23.2_{b}	28.8_{b}	6.28_{ab}	3.04_{a}	2.34_{a}	9.8_{a}	0.18_{a}	24.3_{e}
T5	28.7_{a}	26.6_{c}	6.03_{cd}	2.86_{b}	2.21_{a}	9.6_{a}	0.21_{a}	26.6_{bcd}
T6	26.6_{ab}	27.6_{bc}	6.14_{bc}	2.84_{b}	2.26_{a}	9.4_{ab}	0.16_{a}	26.5_{cd}
T7	16.2_{c}	30.3_{a}	6.35_{a}	3.06_{a}	2.46_{a}	9.7_{a}	0.19_{a}	27.8_{a}
				2008				
T1	29.8_{a}	24.6_{d}	6.04_{f}	2.64_{d}	2.26_{e}	9.4_{ab}	0.16_{a}	27.3_{c}
T2	32.4_{a}	26.7_{c}	6.17_{e}	2.81_{c}	$2.28d_{e}$	9.2_{b}	0.10_{a}	28.7_{abc}
T3	29.9_{a}	27.2_{c}	6.23_{de}	2.92_{bc}	2.34_{cd}	9.6_{ab}	0.16_{a}	28.5_{abc}
T4	18.6_{c}	30.7_{ab}	6.64_{b}	3.17_{a}	2.46_{b}	9.8_{a}	0.20_{a}	25.4_{d}
T5	25.7_{b}	27.4_{c}	6.31_{d}	2.95_{b}	2.15_{f}	9.6_{ab}	0.10_{a}	27.6_{bc}
T6	26.8_{ab}	29.8_{b}	6.48_{c}	2.97_{b}	2.41_{bc}	9.4_{ab}	0.09_{a}	28.8_{ab}
T7	14.7_{c}	32.7_{a}	6.78_{a}	3.21_{a}	2.57_{a}	9.8_{a}	0.24_{a}	29.9_{a}

Values within a row with the same letter are not significantly different ($p<0.05$).

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Table 3. Effect of bagging treatments on fruit harvest dates and harvesting spread during 2007 and 2008.

Treatment	2007		2008	
	Harvest date	Harvesting spread	Harvest date	Harvesting spread
T1	11/10	30	14/10	36
T2	5/10	24	7/10	26
T3	28/9	20	24/9	22
T4	18/9	15	25/9	13
T5	3/10	27	2/10	23
T6	24/9	22	27/9	18
T7	23/9	12	22/9	10

Table 4. Effect of bagging treatments on fruit weight loss, rutab and decay percent after 15 days from cold storage during 2007 and 2008.

Treatment	2007			2008		
	Weight loss (%)	Rutab (%)	Decay (%)	Weight loss (%)	Rutab (%)	Decay (%)
T1	2.20_{b}	11.54_{c}	13.04_{d}	2.21_{c}	14.65_{b}	12.98_{c}
T2	2.12_{b}	9.85_{c}	10.26_{d}	$2.36b_{c}$	13.54_{b}	14.65_{bc}
T3	2.02_{b}	10.65_{c}	19.54_{bc}	$2.30b_{c}$	12.33_{b}	22.46_{ab}
T4	3.43_{a}	22.65_{a}	37.32_{a}	3.04_{a}	25.32_{a}	28.07_{a}
T5	2.15_{b}	11.62_{c}	14.56_{cd}	1.95_{c}	11.41_{b}	8.87_{c}
T6	2.19_{b}	13.47_{bc}	12.56_{d}	2.05_{c}	10.29_{b}	10.86_{c}
T7	2.87_{a}	17.42_{b}	24.47_{b}	2.71_{ab}	20.34_{a}	23.54_{ab}

Values within a row with the same letter are not significantly different ($p<0.05$).

Table 5. Effect of bagging treatments on fruit weight loss, rutab and decay percent after 30 days from cold storage during 2007 and 2008.

Treatment	2007			2008		
	Weight loss (%)	Rutab (%)	Decay (%)	Weight loss (%)	Rutab (%)	Decay (%)
T1	5.12_{c}	18.65_{c}	24.76_{c}	4.76_{de}	23.53_{b}	19.54_{c}
T2	4.42_{c}	20.42_{c}	22.58_{c}	4.54_{e}	20.46_{b}	17.89_{c}
T3	5.07_{c}	17.65_{c}	23.56_{c}	4.36_{e}	17.67_{b}	21.07_{c}
T4	7.51_{a}	39.54_{a}	48.65_{a}	6.21_{a}	40.06_{a}	42.87_{a}
T5	4.77_{c}	16.87_{c}	21.54_{c}	4.24_{e}	16.72_{b}	17.54_{c}
T6	5.18_{c}	18.43_{c}	26.78_{c}	4.92_{bce}	17.65_{b}	18.43_{c}
T7	6.08_{b}	29.33_{b}	34.87_{b}	5.72_{ac}	32.94_{a}	29.87_{b}

Values within a row with the same letter are not significantly different ($p<0.05$).

Phytochemical Profile and Antioxidant Activity of *Phoenix dactylifera* L., *Phoenix canariensis* L. and *Chamaerops humilis* L.

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Keywords: date palm, phytoconstituents, antioxidant activity, bioautography

Abstract

In arid regions, the date palm is the most important plant at the ecological, economic and social levels. In Algeria, many date palm cultivars exist in all traditional palm groves, especially those located in the south-west of Algeria. Each oasis has a composition of its own. The identification of these cultivars is based on fruits' and seeds' morphological description. However, those criterions are problematic.

We propose to characterize phytoconstituents of leaflets from 20 cultivars of date palm in looking for biochemical markers in order to contribute to the cultivars of *Phoenix dactylifera* L.'s inventory. First, we established a phytochemical screening to determine the composition of the cultivars' secondary metabolites. Then, we prepared and analyzed by thin layer chromatography the different extracts to identify substances of secondary metabolism. We revealed and measured phenolic acids, flavonoids and tanins. After the hydrolysis, we identified the aglycones as quercetin and isorhamnetin. The osidic part was identified as galactose. Nevertheless, these metabolites do not allow identifying univocally the tested cultivars. They allow differentiating the two genus we studied *Phoenix* (*dactylifera* and *canariensis*) and *Chamaerops*. Indeed, a strong antioxidant activity was detected in the aqueous extract of date palm cultivars but not in *Chamaerops humilis* L. Some cultivars of the date palm ('Ghars' and 'Adoukli') are as active as the authentic witnesses used such as BHA, ascorbic acid and quercetin.

INTRODUCTION

In arid regions, the date palm is the most important plant at the ecological, economic and social levels. In Algeria, many date palm cultivars exist in all traditional palm groves, especially those located in the South-West of Algeria. The identification of these cultivars is based fruits' and seeds' morphological description. But those descriptors raised a problem due to environmental conditions which vary from one region to the other.

Secondary metabolites can be considered as chimiotaxonomic markers of several classes of vegetals. Ribereau-Gayon (1968) discribed a certain specificity in the repartition of phenolic compounds within the *Malus* and *Pyrus* genus. Otto and Volker (2001) noted that terpenoids are very useful for systematics of conifers.

The work of Ouafi et al. (1988) pointed out that *Phoenix dactylifera* L. is very rich in phenolic compounds (flavonoids and phenolic acids). The aglycone forms of those flavonoids are infraspecific markers and the forms heteroside native constitute markers of nine studied cultivars (Ouafi and Bounaga, 2008).

The aim of this work consists in:

- Establishing the photochemical profile of secondary metabolites (in particular the phenolical compounds) of twenty cultivars of *P. dactylifera* L. The study deals also with another species of *Phoenix* and another genus of the *Arecaceae* family (*P. canariensis* L. and *Chamaerops humilis* L.) to see if phenolic compounds belong to the specie, the genus or the family.
- Study the antioxidant activity caused by the use of date palm in traditional medicine

(Bellakdar, 1997). This activity has been evaluated on leaves of *P. dactylifera* L., *P. canariensis* L. and *C. humilis* L. compared to reference substances (quercetin, ascorbic acid and butylated-hydroxyanisol).

MATERIALS AND METHODS

Plant Materials

The leaflets sampling of date palm was made on about twenty cultivars of (*Phoenix dactylifera* L.) in the wilaya of Adrar, in Algeria, in January 2008 (Table 1).

The leaflets of two other *Arecaceae* (*Phoenix canariensis* L. and *Chamaerops humilis* L. were taken from the wilaya of Oran, in Algeria.

The vegetable material was identified by the National Research Institute of Agronomy, in Algeria.

The leaflets are cleaned with alcohol in order to eliminate any trace from cochineal and are dried with the drying oven at 45°C during 48 h. The dried material is crushed using a crusher with metallic beads.

The vegetable powder of each sample was kept in bottles in the dark until it was used.

Preparation of Plant Extracts

The aqueous extract was prepared by refluxing (60-70°C), 10 g of plant material with 100 ml of distilled water, for 30 min. This was repeated three times with fresh solvent each time, followed by filtration. Filtered extracts were mixed and lyophilized to dryness.

The samples are prepared with a concentration of 2 mg ml^{-1} in MeOH, and then homogenized, using an ultrasonic bath until complete dissolution.

Phytochemical Screening

For each aqueous extract, the main chemical components were characterized by the establishment of their chromatographic profiles on a thin layer (TLC) on silica gel plates 60 F254, 0.2 mm.

The solvent systems are: AcOEt-HCOOH-AcOH-H_2O (100:11:11:26) (phenolic acids, flavonoids, coumarins, sesquiterpens lactons and triterpenes), AcOEt-MeOH-H_2O (100:13,5:10) (anthracenic derivatives, anthrones et anthranols, cardiotonic glycosides), $CHCl_3$-MeOH-H_2O (70:30:4) (lignans), AcOEt-MeOH-H_2O (100:17:13) (free quinons), $CHCl_3$-MeOH-H_2O (64:40:8) (saponins) (Wagner and Bladt, 1996).

To detect the metabolites the chromatograms were revealed under UV at 254 nm, 365 nm and visible light before and after they were sprayed. The reagents used are: Folin-Ciocalteu (phenolic acids), KOH (anthracenic derivatives, anthrones and anthranols, coumarins and free quinons), Neu (Flavonoids), $SbCl_3$ (cardiotonic glycosids and lignans), Zimmermann (sesquiterpens lactons) and anisaldehyde (triterpens) (Wagner and Bladt, 1996).

The tanins have been highlighted by a colored reaction with $FeCl_3$ (Dohou et al., 2003).

Acid Hydrolysis

The acid hydrolysis is carried out on some vegetable powder mg using (2N)HCl-MeOH (1:1) in glass tubes sealed and placed in a drying oven at 100°C during one hour. After cooling, the aglycon fraction is separated from the osidic fraction by an extraction liquid-liquid against dichloromethane (CH_2Cl_{22}).

The resulting organic phase is evaporated dry, then dissolved in few ml of methanol.

The released aglycons are identified by TLC in toluene-AcOEt-HCOOH (50:40:10) then revealed by NEU's reagent. The aqueous phase containing the polar compounds, in occurrence monosaccharid neutralized by successive evaporations

(3 times) in presence of methanol, will be used for the analysis of sugars in $CHCl_3$-MeOH-H_2O (64:40:8) revealed by a solution of DPAP (acid diphenylaminophosphoric) followed by a heating.

Determination of Total Phenolic Compounds

The content total polyphenols is estimated by the method of Folin-Ciocalteu (Singleton and Rossi, 1965) with some minor modifications. 100 µl of plant extract are added to 500 µl of reagent of diluted Folin-Ciocalteu (1/10 dissolved in distilled water). The mixture is agitated by vortex and is left in darkness during 5 min and room temperature. Then 1.5 ml of saturated sodium carbonate (2% dissolved in distilled water) are added with agitation. After incubation during one hour, the reading of the absorbance is carried out with the spectrophotometer UV-Visible (8500 P Double-BEAM spectrophotometer) with 765 nm against a prepared white, by replacing the extract with methanol. The quantities of total phenolics are expressed as milligrams of gallic acid equivalents (GAE) per gram of freeze-dried extract (mg GAE g^{-1} of freeze-dried extract) to starting from a linear calibration curve, prepared using gallic acid with various concentrations (4.76, 9.52, 14.2, 19 and 23.8 µg ml^{-1}) under the same conditions as the sample.

Determination of Total Flavonoids

The total flavonoid content of the extracts was determined by colorimetrically using the method described by Kim et al. (2003) with some modifications. With 500 µl of plant extract, one adds 1500 µl distilled water. In time zero, 150 µl $NaNO_2$ 5% µl (dissolved in distilled water) are added. After 5 min 150 µl of a solution of $AlCl_3$ with 10% (dissolved in MeOH) are added. After 11 min 500 µl NaOH (1 M, dissolved in distilled water) are added. The mixture is agitated with the vortex, the reading of the absorbance is carried immediately to 510 nm. Total flavonoids content was calculated as a calibration curve using catechin (5, 10, 15 and 25 µg ml^{-1}) as standard and expressed as milligrams of catechin equivalents (CE) per gram of extract freeze-dried (mg CE g^{-1} of freeze-dried extract).

The experiment was done in triplicate.

Evaluation of Antioxidant Activity Using the DPPH Method

TLC bioautography for antioxidant activity was determined using the DPPH-test according to Gu et al. (2009). We deposited the same quantities (25 µl at 2 mg ml^{-1}) of each extract on the plate of silicagel 60 F254. TLC plates were developed in AcOEt-HCOOH-AcOH-H_2O (100:11:11:26). After the migration and drying of chromatograms, the plates are pulverized with 0.2% DPPH in methanol. Bands with the DPPH scavenging activity were observed as white yellow bands on a purple background.

All samples were analyzed in three replications.

The conventional spectrophotometric DPPH scavenging capacity assay was conducted as described by Blois (1958) with minor modification.

Briefly, an aliquot 50 µl of different concentrations of each extract (10, 20, 30, 40, 50 µg ml^{-1}) was added at 1950 µl methanolic solution of DPPH (6.10^{-5} M). The mixture was incubated at room temperature in darkness for 1 h. The absorbance was then measured at 517 nm and corresponds to the extract ability to reduce the radical DPPH to the yellow-coloured diphenylpicrylhydrazine. The antiradical activity was expressed as IC50, the antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was estimated percentage inhibition (PI) and calculated using the following equation:

$$PI=[(A_0-A_S)/A_0]\times 100 \quad (1)$$

where A_0 is the absorption of the negative control solution (containing only DPPH), A_S is the absorption in the presence of the plant extract in DPPH solution. The ascorbic acid,

quercetin and the BHA were used as the positive controls.

The results are expressed by the means of three measurements ± standard deviation.

RESULTS

Photochemical Screening of Extracts

After observing chromatograms under UV at 254 and at 365 nm before and after revelation by the specific reactive of each constituent of secondary metabolism, and the calculation of their Rf (Table 1), we observed the presence of:

Phenolic Acid

A spot of blue fluorescence (Rf 0.56) in all aqueous extracts in *P. dactilyfera* L., *P. canariensis* L. and *C. humilis* L.

Flavonoids

Several spots of diverse fluorescence (yellow, orange and green) (Rf 0.35, 0.45, 0.50, 0.73, 0.91) common to the set of cultivars of *P. dactylifera* L. and *P. canariensis* L.

A supplementary layer of blue fluorescence and Rf 0.62 is seen in the cultivar of 'Tinnaceur' and of Rf 0.97 within 'Bouarif', 'Warglia' and *P. canariensis* L.

The profile of flavonoids of *Chamaerops humilis* L. is very different from what is observed in *P. dactylifera* L. and *P. canariensis* L. with three spots of blue fluorescence (Rf 0.05, 0.56, 0.62), among them two yellow-green fluorescence (Rf 0.56), and a yellow-orange fluorescence with a weak intensity (Rf 0.73).

Identification of Aglycon

After observation of acid hydrolysats under UV at 254 and at 365 nm before and after revelation of the Neu reactive we observed that:

- the hydrolysats of *P. dactylifera* L., *P. canariensis* L. and *C. humilis* L. show a spot of the same Rf 0.52, corresponding to quercetin.
- the extracts of *P. dactylifera* L. present a supplementary spot of Rf 0.61 corresponding to isorhamnetin.

Identification of Sugars

Acid hydrolysis has detected a spot of Rf 0.37 corresponding to galactose in extracts of *P. dactylifera* L., *P. canariensis* L. and *C. humilis* L.

Detection of Tanins

Positive result on the set of extracts, the reaction with $FeCL_3$ gives a blue-black precipitate corresponding to tanin. However, coloration is very weak in *C. humilis* L.

Other Secondary Metabolites

The reactions of putting into evidence by TLC of free quinons, coumarins, lignans, anthracenic derivatives, sesquiterpens lactons, saponins and cardiotonic glycosides in the aqueous extracts of *P. dactylifera* L., *P. canariensis* L. and *C. humilis* L. were negative. Two possibilities, either they are absent or their low levels cannot be detected with the chromatographic techniques used.

Total Phenols

The total phenolics contents of the studied extracts vary greatly and are between 57.94±3.13 and 170.79±2.39 mg of GAE g^{-1} of freeze-dried extract in the leaves of *P. dactylifera* L. (Table 2).

The cultivars 'Ghars', 'Deglet bouda', 'Aharthan', 'Adoukli', 'Baouarif', 'Aghamou', 'Aghares' and male date palm ('Doukar') are the richest in total polyphenols (above 100 mg GAE g^{-1}).

The other cultivars, 'Bamakhlouf', 'Takerbucht', 'Baouadhim', 'Warglia', 'Timjuhert', 'Timliha', 'Tgaza', 'Tinnakour', 'Tinnaceur', 'Deglet mahmoud', 'Deglet nour', have inferior values at 100 mg GAE g^{-1}, the same as *Phoenix canariensis* L. In opposition, *Chamaerops humilis* L. is the poorest in polyphenols, 25.20±0.46 mg GAE g^{-1} of freeze-dried extract.

Flavonoids

The total flavonoids varied from 24.25±0.71 to 69.07±2.68 mg CE g^{-1} freeze-dried extract in the leaves of *P. dactylifera* L. (Table 2).

The cultivars 'Ghars', 'Aharthan', 'Deglet bouda', 'Adoukli', 'Aghamou' have the most important contents of flavonoids, higher than 50 mg CE g^{-1}. The other cultivars of palm date as *Phoenix canariensis* L. have a weak content. *Chamaerops humilis* L. has a very low content of 17 mg CE g^{-1} of freeze-dried extract.

'Takerbucht', a resistant cultivar to *Fusarium oxysporum* f. sp. *albedinis* has a higher content of polyphenols and flavonoids than the sensitive cultivar 'Deglet nour' (Table 2).

Antioxidant Activity

The results by TLC bioautography for antioxidant activity gave several anti-radical spots (yellow on a purple base) in all aqueous extracts (*P. dactylifera* L. and *P. canariensis* L.) except for *Chamaerops humilis* L. which presents just one task with very weak intensity (Fig. 1).

DPPH Reduction Assay

Values of IC_{50} express the concentration of extract necessary to diminish the absorbance of DPPH of 50% (inhibition of DPPH at 50%). The values IC_{50} are represented in Table 2.

For the aqueous extracts of *P. dactylifera* L., values IC_{50} vary from 13.15 to 75.46 µg ml^{-1}. The 'Ghars' cultivar has an IC_{50} 13.15 µg ml^{-1}, inferior to IC_{50} of synthetic antioxidant widely used in agro-alimentary industry: quercetin (IC_{50} 13.52 µg ml^{-1}, ascorbic acid (IC_{50} 14,37 µg ml^{-1}) and BHA (IC_{50} 17.90 µg ml^{-1}). Likewise, 'Adoukli' is IC_{50} (16.39 µg ml^{-1}) inferior to the referent substance BHA. Those two cultivars present a very strong antioxidant activity which can be explained by the presence of polyphenols with a proven antioxidant activity. On the contrary, *Chamaerops humilis* L. shows a very weak antioxidant activity (94,55 µg ml^{-1}) which is confirmed by the bioautographic method. It has a weak content of polyphenols (25.20 mg GAE g^{-1}) and flavonoids (17.26 mg CE g^{-1}).

DISCUSSION

The TLC phytochemical confirms the wealth in phenolic compounds in the leaflets of date palm observed by Ouafi et Bounaga (2008). Those authors identified the aglycone flavonics as being flavonols (quercetin and isorhamnetin) identified as well by Ziouti et al. (1996) and flavons (luteolin, tricine and chrysoeriol) by the HPLC method. Ouafi and Bounaga (2008) think that aglycone flavonics are specific markers of *dactylifera* species.

According to our results the quercetin seems to exist in *P. canariensis* L. and *C. humilis* L. Only isorhamnetin seems to be present in the species *P. dactylifera* L.

Results show that flavonoids are heterogeneous within cultivars of palm date, but without being able to find out phenolic markers of each cultivar. It was not possible to validate the role of phenolic compounds in the resistance of palm date to bayoud from the qualitative and quantitative viewpoint (Ziout et al., 1996). In effect, contents higher in phenolic compounds has been underlined in several sensitive cultivars ('Ghars', 'Degla Bouda', etc.).

However, the flavonic profil separates the *Phoenix* from the *Chamaerops* genus.

Work on the evaluation of antioxidant activity at the level of leaves of palm date

may not exist but many works on fruit (dates) of date palm have revealed a strong antioxydant activity (Mansouri et al., 2005; Al-Farsi et al., 2007; Biglari et al., 2008; Behija Saafi et al., 2009). The therapy virtues of dates are numerous (Benchelah and Maka, 2006; Belguedj et al., 2008).

According to enquiries on the field, leaflets of date palm are also used against certain diseases (essentially ocular afflictions and facilitate cicatrisation).

Bellakhdar (1997) reports also the use of *P. dactylifera* L. and *P. canariensis* L. in traditional medicine.

The antioxidant activity of extracts varies and reveals that certain cultivars of date palm as active as the authentic witness used, in the case of 'Ghars' which presents the weakest IC_{50} (and with the strongest activity) then 'Baouarif' whose activity is sensitive and similar to BHA (as a referent). 'Ghars' is the cultivar which contains the highest total polyphenols and flavonoids. As for 'Ghars' it is the cultivar in which the different parts of the plant are the most used in traditional medicine, in certain regions of the Algerian south.

This strong activity shows that the aqueous extract contains substances reacting to the radical DPPH. This activity can be explained by the presence of polyphenols and flavonoids whose antioxidant activity of plants, and the presence of natural substances, mainly polyphenols , with therapeutic components, is well extablished (Dominguez et al., 2005; Pereira et al., 2007; El Gharras, 2009; Gregoris and Stevanato, 2010).

But those results are unsufficient to be able to validate the use of *P. dactylifera* L. in traditional medicine.

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Tables

Table 1. Results of phytochemical screening of plant extracts.

Aqueous extracts	Number of spots	Fluorescence and Rf after revelation at 365 nm	Nature of phytoconstituents
Date palm cultivars			
Baouadhim	6	FYO (0.35); FYO(0.45); FYG(0.50); FYO (0.73); FYG(0.91); FB(0,56)	Flavonoids phenolic acid
Timjuhert	6	id.	id.
Bamakhlouf	6	id.	id.
Adoukli	6	id.	id.
Aghamou	6	id.	id.
Tgaza	6	id.	id.
Aharthan	6	id.	id.
Timliha	6	id.	id.
Ghars	6	id.	id.
Tinakour	6	id.	id.
Deglet nour	6	id.	id.
Takerboucht	6	id.	id.
Aghares	6	id.	id.
Deglet mahmoud	6	id.	id.
Deglet bouda	6	id.	id.
Tinnaceur	7	FYO(0.35); FYO(0.45); FYG(0.50); BF(0.62);FYO(0.73); FYG(0.91); BF(0.56)	id.
Baouarif	7	FYO(0.35); FYO(0.45); FYG(0.50); BF(0.73); FYG(0.91); BF(0,97) BF(0.56)	id.
Warglia	7	id.	id.
Doukkar (male)	7	id.	id.
Other *Arecaceae*			
Phoenix canariensis L.	7	FYO(0.35); FYO(0.45); FYG(0.50); BF(0.73); FYG(0.91); BF(0.97) BF(0.56)	id.
Chamaerops humilis L.	6	BF(0.05); FYG(0.21); FYG (0.41); BF(0.62); FYO(0.73) BF (0.56)	id.

BF: Blue Fluorescence, FYG: Fluorescent Yellow-Green, FYO: Fluorescent Yellow-Orange, Rf: Retention factor

Table 2. Total phenolics contents, flavonoids and antioxidant activity contents of plant extracts.

Aqueous extracts	Total phenolics	Flavonoids	IC_{50}
Date palm cultivars			
Ghars	170,79±2,3	69,07±2,68	13,15±0,27
Deglet bouda	143,12± 2,60	57,15±2,16	26,61±0,45
Aharthan	142,90±10,32	66,19±3,39	24,09±0,07
Adoukli	135,16±1,88	59,41±1,55	16,39±0,07
Baouarif	123,41±4,49	48,10±2,136	18,73±0,23
Aghamou	120,00±2,03	52,42±1,63	18,39±0,35
Aghares	104,68±4,02	43,78±1,23	35,96±0,40
Takerboucht	98,55±3,49	48,10±2,13	32,92±0,66
Bamakhlouf	95,53±4,41	48,31±0,35	23,13±0,75
Baouadhim	87,95±1,85	28,57±1,88	31,25±0,55
Warglia	82,37±4,41	40,91±1,98	27,59±0,71
Timliha	79,73±4,18	29,80±1,55	28,88±0,38
Timjuhert	79,73±3,00	27,75±1,23	29,48±0,56
Tgaza	79,44±1,42	27,95±1,98	31,97±0,57
Tinnakour	73,77±3,62	36,59±0,94	31,30±0,48
Tinnaceur	71,82±2,80	35,56±0,35	32,07±0,16
Deglet mahmoud	68,96±1,71	26,52±1,06	75,46±1,07
Deglet nour	57,94±3,13	24,25±0,71	67,80±1,20
Doukkar (male)	122,22±3,94	53,65±1,85	27,12±0,17
Other *Arecaceae*			
Phoenix canariensis L.	98,76±0,46	44,20±0,94	45,90±0,63
Chamaerops humilis L.	25,20±0,46	17,26±0,61	94,55±3,42
Standards	IC_{50}		
Quercetin	13,52±0,12		
Ascorbic acid	14,37±0,05		
BHA	17,90±0,13		

Total phenolics contents expressed as mg gallic acid equivalents g^{-1} of freeze-dried extract.
Flavonoids contents expressed as mg catechin equivalents g^{-1} of freeze-dried extract.
Antioxidant activity IC_{50} expressed as µg ml^{-1}.

Figures

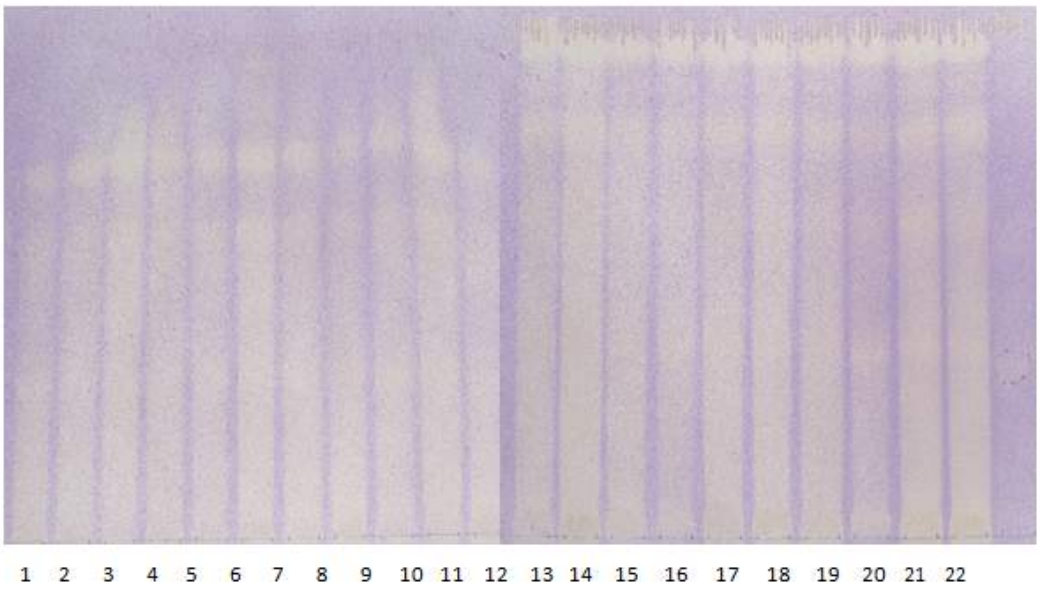

Fig. 1. TLC plates stained with 0.2% DPPH solution in methanol and visualized under visible light. 25 microliters (2 mg ml^{-1}) of each extract was applied as bands on TLC layer. Bands with the DPPH scavenging activity were observed as white yellow bands on a purple background.
1. Baouadhim, 2. Timjuhert, 3. Bamakhlouf, 4. Warglia, 5. Tinnakour, 6. Adoukli, 7. Aghamou, 8. Aghamou (Bayoud), 9. Tgaza, 10. Baouarif, 11. *Phoenix canariensis* L., 12. Timliha, 13. Ghars, 14. Tinnaceur, 15. Deglet nour, 16. Takerbucht, 17. Doukar, 18. Aghares, 19. Deglet mahmoud, 20. Deglet bouda, 21. Aharthan, 22. *Chamaerops humilis* L.

Enhancing the Performance of Doka of 'Dhakki' Date Exercising Freeze Technology

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Keywords: 'Dhakki' dates, *Phoenix dactylifera*, freezing, potassium alkali treatment, quality, curing/drying, Pakistan

Abstract

The possibility of employing deep freeze technology was explored so as to enhance the performance of 'Dhakki' date cultivar at the doka stage. The fruits harvested at the fully matured hard, yellow and astringent stage were stored at 10, 0 and -15°C for one year, and examined for hardness, visual appearance and mold resistance. Subsequent performance of the doka was evaluated in terms of curing/drying period, quality, yield, and shelf stability of the product at the accelerated temperature of 40°C. The doka maintained at 10°C became mold infested just after 3 months storage, while those at the lower temperatures remained sound during the study period. Moreover the doka on thawing developed characteristics similar to the dong form resulting in color change from yellow to golden brown, sweeter in taste, and softer in texture. Subsequent curing/drying caused the product's patchy and loose skin with gritty mouth feel, the feature however slightly improved on lowering the freeze temperature. Treatment with the optimum doze of potassium hydroxide introduced a product with enhanced quality, yield, and stability. Leaving doka frozen below 0°C until required for further processing additionally offers sound proposition for streamlining the availability of fresh dates for an extended period besides reducing post-harvest losses, facilitating the marketing and bringing price stability of dates.

INTRODUCTION

The role of date palm (*Phoenix dactylifera* L.) has been well established ever since the birth of the human race. The Holy Qura'n commended dates at several places, and hence the dates became part of Islamic culture. Considering it as a virtue Muslims consume dates at several religious occasions. Besides, the date palm nourishes millions all over the world and contributes significantly towards their development and prosperity particularly to those living in the Arabic world. Date fruits being sweet and palatable, are consumed as a staple diet supplying 2500-3000 K calories/kg of physiological energy. The fruit provides several vitamins, a high amount of potassium, and furnishes overall highest percentage of easily assimilating carbohydrates. Further it contributes a large amount of cellulose and hemi-cellulose materials necessary for maintenance of intestinal motility.

The date fruit is an important cash crop for Pakistan, and a good source of foreign exchange earnings. The total cultivated area of all types of dates in Pakistan exceeds 78.1 thousand hectares with its estimated annual production over 630 thousand tons constituting about 11% of total world production (Anon., 2002). According to the Food and Agriculture Organization (FAO), Pakistan is 4th world largest date producing country (Anon., 2000), exporting dried dates worth US$ 17.8 million annually (Anon., 2001). Cultivation of dates in North West Frontier Province (NWFP) is over 1000 ha with 6700 tons production, and Dera Ismail Khan alone contributes more than 50% of it (Anon., 2002).

'Dhakki' being the most promising local cultivar of Dera Ismail Khan is amongst the few top world-leading cultivars. It furnishes about 60% of the total share of this region and

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displaying continuous increases in its production. The 'Dhakki' has fruits of choice and is market oriented, and hence is gaining unprecedented importance both in domestic and foreign markets. The fruit possesses large size (4-5 cm long and 2-3 cm thick) and heavy in weight (16-20 g/fruit), very fine texture with exceptionally high flesh/stone ratio (96%), relishing taste, good appearance, and reasonably longer shelf life (Baloch, 1999). In spite of the fact that the 'Dhakki' date is a crop of national significance, it is not gaining requisite pace for further development. 'Dhakki' being a late cultivar is facing diversified problems, some of them are dependent upon the weather stresses while the other arise out from resource shortage and lack of know-how. The ripening season of the date starts during high summer temperatures of 40-50°C with peak production in August-September. This period unfortunately coincides with monsoon season in Pakistan while commissioning of the Chashma Right Bank Canal has made the climate even more variable and unpredictable. Since 'Dhakki' fruit at full mature/ripened stage is relatively more susceptible to hot and humid conditions, it receives substantial damages from monsoon rain and storm. Moreover, the fruit during this period is at eatable (doka/dong) stage and prone to infestation by insects/birds and diseases and hence is invaded at a rapid rate under the favorable climate of relatively reduced temperature with high humidity. The losses continue pilling up so long as fruits stay on-tree for want of dong formation until the end of September. The fall in day temperature on account of the ending summer season slows down on-tree fruit ripening; consequently the period between consecutive pickings as well as the number of pickings is increased amounting to further infestation and expenses. At the same time, a large quantity of freshly ripened dates from other cultivars becomes available, which not only gluts local market but pre-occupies orchard space thus proper handling and processing becomes difficult of 'Dhakki' date, and the surplus produce is wasted. Under the prevailing detrimental environment that expands over weeks the 'Dhakki' date inflicts enormous crop losses and quality degradation. Such losses cannot significantly be reduced due to lack of appropriate on-farm shelters, or other facilities for keeping the produce safe.

The traditional methods for ripening/curing are still under practice, and the fruits at late dong stage are spread on mats and exposed to sun in open air. The sun drying under dusty environment renders the product non-uniform and substandard. Due to persistent raining and stormy conditions large amounts of the harvested dates get moldy, fermented, dusty, and birds/insects invaded. Thus a colossal amount of fruit wastage wrecks the crop yield and ultimately the economy of the growers becomes devastated. In our previous studies on ripening of 'Dhakki' dates applying microwave energy (Saleem et al., 2002; Baloch et al., 2003) and other artificial means (Saleem et al., 2005) a successful conversion of doka into tamar has been demonstrated. Such techniques shortened the period of fruit on-tree by 3-4 weeks, curtailed curing period appreciably, increased yield substantially, and enhanced quality of the product significantly.

In order to maintain the current tempo of cultivation and propagation of the 'Dhakki' cultivar it is imperative to make 'Dhakki' a profitable crop. Since the freshly cured form of the date is much liked by the consumers and attracts higher prices, attention therefore is focused to ensure the availability of fresh dates as and when required. Except the storage at reduced temperature, no effective long-term preservative methodology is apprehended for storage at perishable doka stage. Keeping the whole scenario in view, the present study was therefore carried out to explore the potential of freeze technology so as to make fresh doka available over an extended period for on-demand production of fresh dates, and further to improve maturation performance, and enhance quality, yield and stability of cured/dried product on storage in summer temperatures.

MATERIAL AND METHODS

The mature 'Dhakki' fruits of early doka stage fairly astringent in taste having a hardness index of 295-300 mm Hg cm^{-2} and yellow in color were harvested from the orchard of the Agriculture Research Institute, Dera Ismail Khan, and subjected to processing, storage and quality evaluation as per scheme of Figure 1. Briefly the dokas, after being given a thorough washing with tap water and wiping off excess liquid, were

taken in 250-g polyethylene bags and stored at temperatures of 10, 0 and -15°C for a period of 12 months. For this purpose a space was reserved in the cold storage maintained at 10°C, whereas the fruits meant for storage at 0 and -15°C were kept in a plate type house-hold deep freezer (Triplet Deep Freezer, provided by Waves Company, Ltd. Pakistan). The samples were taken out periodically from the storage to examine hardness, microbial growth, and surface appearance. Fruit hardness was measured by a hardness testing device developed previously (Baloch et al., 2003). Observing slime formation on the fruit surface, given peculiar fermenting smell or recording other sensory characteristics ascertained the fruit quality. In order to evaluate performance of the frozen doka for successive maturation operations the samples after thawing were subjected to ripening/curing/drying using an air overflow cabinet drier fitted with a temperature/humidity control unit. Initially the temperature and humidity were set respectively at 42±2°C and 75-80% for 24 h and then the temperature increased to 50±2°C and humidity reduced to 50-55% until the moisture content reduced to 25-26%. The period for curing/drying, the extent of ripening/yield percentage and other quality attributes were determined. Freshly harvested doka of the same maturity (with or without overnight freeze treatment) were also processed side by side to serve as controls. Further studies were conducted to verify the effectiveness of potassium hydroxide treatment against loosening of fruit skin as observed during preceding experiments. The doka from 6-months storage at -15°C after being thawed were treated with 0.1 to 1.0% (g/ml) potassium hydroxide by steeping the fruits in the solution (1:1 w/v) for 1 m at 40±1°C. The treated fruits after being given a water rinse were cured/dried under above specified conditions. To examine shelf stability of the product the cured samples taken in polyethylene bags were incubated in a thermostatically controlled oven at 40°C for 8 months, and quality evaluated. Moisture contents were determined by the oven method of the AOAC (1998) and the extent of ripening/curing and yield was determined according to Baloch et al. (2003).

Quality of the products was evaluated using the standard organoleptic technique for sensory scoring through trained panelists (Jellinek, 1985). A descriptive panel was composed of individuals to evaluate overall acceptability of the products keeping in view quality parameters like skin appearance, grit, taste, color, and shine of the fruits. The samples were scored on hedonic scale, 1 for not acceptable and 10 for highly acceptable product. The data were analyzed statistically using MSTAT-C version 2-10 software package applying Completely Randomized Design (CRD). The means are separated by LSD test using the same package.

RESULTS AND DISCUSSION

The doka showed no signs of deformity on storage at 10°C for at least 3 months; however, most of the fruits beyond that period gave a dull look with soft patches on the surface, and increased variation in firmness. The fruits became shriveled and mold invaded (Tables 1, 2 and 3). Whereas, the doka kept for 3 months at 0 and -15°C displayed much soft and tender texture on thawing with significantly reduced hardness index. Some of the fruits from 0°C developed brown spotting possibly caused by crystallization of soluble solids. However, no signs of mold growth were evident up to 12-months at both of the temperatures. The fruits stored at -15°C turned out more soft and juicy on thawing, and were found completely devoid of astringency and sweet in taste, very attractive, and had developed a desired golden amber color matching the characteristics advanced on natural ripening (Table 3). This confirms our previous findings that the frozen doka converts into dong-like product on thawing (Baloch, 1999). We agree with views of Hussan (1989) who reported a marked activity of ripening enzymes including polyphenol oxidase and pectin methyl esterase after dates were thawed. Maximum polyphenol oxidase activity was recorded after 3 h thawing in ‘Zaghloul’ and ‘Bent-Aisha’, and after 18 h in ‘Samari’ date. Moreover, the bitterness of the doka became precipitated, and the astringent fruit tasted sweet. El-Din (1998) also observed that the khalaal stage of ‘Bent Aisha’ date by freezing at -18°C developed to rutab (soft) stage. He further reported that the freezing

method gives the best quality in which the low molecular weight tannin contents were decreased.

Ripening yield of the product from curing/drying was improved on storing the samples at each selected low temperatures. The samples for 3 months storage at 10°C exhibited 1.2 times greater yield on subsequent curing/drying than from those harvested fresh (65% yield, Table 4). Whereas those stored at 0 or at -15°C rendered a product with much higher yield up to 95% (Table 4). A short overnight freeze treatment also proved as useful in improving product yield. However, the ripening yield declined for samples beyond 6 months storage at -15°C. Since the fruits were of early doka stage possessing thick and hard skin that loosened off from the pulp as a result of freezing/thawing. The product manifested cracked skin on curing/drying. The extent of the cracking was higher in the samples that were left frozen at the lowest temperature. The study indicates that freezing/thawing probably affected the fruit pulp and its contents more severely than its skin. It is speculated that the ice crystals formed on freezing ruptured the fruit cells which on thawing allowed the ripening system to function rapidly in the fruit pulp. Besides improvement in the quality and yield the freeze technology shortened the requisite operational time for the subsequent curing/ripening process from days to hours reducing the moisture content of the product to 24-26% within 72 h. Whereas the freshly harvested doka used as a control took 7-8 days under similar curing conditions (Table 4). The selected level of moisture contents corresponded to 0.62 a_w at which the samples showed maximal stability on subsequent storage at 40°C (Baloch et al., 2005).

The frozen doka after curing unexpectedly were underscored for giving a gritty mouth-feel caused by the broken hard skin pieces. Preliminary study was therefore initiated with a view to weaken the loosened hard skin as a result of thawing and subsequent curing/drying. Utilizing the chewing property of alkali to polysaccharides a treatment consisting on potassium hydroxide instead of sodium hydroxide was chosen for the purpose so as to maintain composition of the date product sodium free for natural resemblance. Observing improvement in quality of the product by the preliminary treatment with potassium hydroxide (0.50 g/100 ml) the study was further conducted using different concentrations of potassium hydroxide in the range of 0.1-1.0% (g/ml). The thawed fruits from frozen storage were allowed to steep in the reagent solution for 1 min at 40°C. The treated fruits were cured/dried to 25% moisture contents, and quality evaluated. In order to find out stability the product was further stored for 8 months at the prevailing summer temperature of 40°C, and the product was evaluated for acceptability on the basis of taste, appearance and mouth-feel considering grittiness as a major factor. Improvement in quality was noted with increasing the treatment concentration up to 0.25%. The product tended to become dark and the score declined gradually on further increase in the concentration causing the product to become unacceptable with 1.0 g/100 ml concentration. The treatment concentration of 0.25% was indicated as optimal by receiving the highest score of 9.0-9.8 (Table 5). The sample also displayed enhanced storage stability at 40°C, besides giving much increased yield of 98% (Table 4). The study certainly conceives a great breakthrough for the date processing industry as it furnishes a product with exceptionally high yield of best quality and stability. Moreover, the process offers at least 3 weeks saving from on-tree period and prevents expected great damages caused by various environmental factors. The development (Fig. 1) further ensures streamlining the supply of a uniform product to the market. The consumption and marketing of quality dates and date products would become controlled, and hence the prices kept greatly stable. Moreover, it guaranties off-season and on-demand supply of freshly ripened dates at least for 6 months, and keeps marketing activities progressing almost year round. Although the economics have not been worked out it is highly likely to get a much higher net return as a result of substantial reduction in overall losses, better price recovery and conceding extended market activities. The technology offers a sizable breathing space to manage harvesting, processing and storage of dates while minimizing the date glutting. The option would certainly help boosting up the economy of the growers/farmers and the country at large.

CONCLUSIONS

The harvested doka of 'Dhakki' dates sustains a varying degree of textural and biochemical changes on prolonged freezing, however, it remains safe and free from mold growth at 0 and -15°C for at least a year. The doka left frozen at -15°C turns into dong form by acquiring characteristics similar to those advancing on natural ripening that lead to tenderness, astringency precipitated and golden amber color developing. The fruits can be cured/dried at a faster rate to a cured date-biomass. However, the product acquires a chipped surface due to skin peel off. The undesirable surface feature is adequately corrected after given a mild potash alkali treatment consequently the product improved quality, yield and storage stability at 40°C. The process envisages great potential to ensure the availability of fresh dates at off-season. Further it will monitor the production, maintain the price of the product stable and keep economic and marketing activity of dates alive, and expected sizable reduction in post harvest losses.

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Tables

Table 1. Ranges of hardness index (mm Hg cm^{-2}) of 'Dhakki' doka during 12-months storage at reduced temperatures.

Storage temperature (°C)	Storage period (months)				
	0	3	6	9	12
10	300-295 (298A)[1]	295-260 (278A)[1]	270-190 230B)[1]	265-100 (183C)[1]	240-40 (140D)[1]
0	300-295 (298A)[1]	70-50 (60E)[1]	30-20 (25F)[1]	-	-
-15	300-295 (298A)[1]	20-10 (15F)[1]	20-10 (15F)[1]	-	-

[1] Mean values bearing different letters (A-F) in each column differ significantly (LSD, P≤0.05).

Table 2. Appearance of mold growth on 'Dhakki' doka stored for 12 months at reduced temperatures.

Storage temperature (°C)	Storage period (month)				
	0	3	6	9	12
10	ND	ND	SD	FD	F
0	ND	ND	ND	ND	ND
-15	ND	ND	ND	ND	ND

ND (not detectable), SD (slightly detectable), FD (fermentation detectable), F (fermented).

Table 3. Visual appearance of 'Dhakki' doka during 9-months of storage at reduced temperatures.

Storage temperature (°C)	Storage period (months)			
	0	3	6	9
10	Translucent yellowish, hard	Dull yellowish, slightly wrinkled, firmness variable with patches	Dull yellowish, slightly wrinkled, firmness variable with patches, mushy and slimy	Dull, fairly wrinkled, firmness variable, microbe invaded
0	Translucent yellowish, hard	Yellowish dong-like, slightly soft, skin slightly spotted, sweet in taste	Yellowish dong-like, soft, skin slightly spotted, sweet in taste	Yellowish dong-like, soft, skin spotted, sweet in taste
-15	Translucent yellowish, hard	Golden amber, soft dong, very attractive, bright, sweet and juicy	Golden amber, soft dong, very attractive, bright, sweet and juicy	Golden amber, softer dong, very attractive, bright, sweet and juicy

Table 4. Effect of freezing of doka and pot. alkali treatment on period of curing/drying, yield and quality of 'Dhakki' date.

Samples*	Curing/ drying period	Ripening/ Yield (%)	Acceptability (score)	Remarks
Doka[1]	7-8 days	65	6.2	Variable quality, less attractive
Doka[2]	80 h	90	8.9	Attractive, sweeter taste, loose skin, patchy
Doka[3]	6 days	80	9.0	Acceptable, but variable in quality
Doka[4]	72 h	92	9.2	Attractive, skin slightly chipped
Doka[5]	72 h	95	8.5	Attractive with luster, skin balloon like and patchy
Doka[6]	72 h	96	8.9	Very attractive, bright and skin mostly intact

[1] Doka freshly harvested, served as a control.
[2] Doka freshly harvested and remained frozen overnight.
[3] Doka left over for 3 months at 10°C.
[4] Doka left over for 3 months at 10°C and remained frozen overnight.
[5] Doka taken out after 6 month's storage at -15°C.
[6] Doka taken out after 6 month's storage at -15°C and treated with 0.50 g/100 ml potassium hydroxide.
*Mean of three readings, ranking value ranges from 1-10, 10 being a highly acceptable sample.

Table 5. Overall effect of pot alkali on acceptability of 'Dhakki' dates with 25% moisture.

Potash alkali conc. (%)	After curing/drying		After 8-months storage at 40°C	
	Score*	Remarks	Score*	Remarks
F+0.0	8.6	Good taste, loosened skin	7.1	Good taste, skin slightly broken
F+0.1	8.7	Good taste, loosened skin	7.3	Good taste, skin slightly broken
F+0.25	9.8	Good taste, very bright amber color, skin intact	8.2	Good taste, bright amber color, skin mostly intact
F+0.35	9.0	Good taste, amber color, skin intact	7.4	Agreeable taste, brown coloration, skin intact
F+0.5	8.9	Acceptable taste, slightly brown color	6.0	Taste like burnt sugar, brown color
F+1.0	7.5	Dark brown	3.2	Taste like burnt sugar, Dark in color

F = Sample left frozen for 6 months at -15°C.
*Ranking value ranges from 1-10, 10 being the most acceptable sample.

Figures

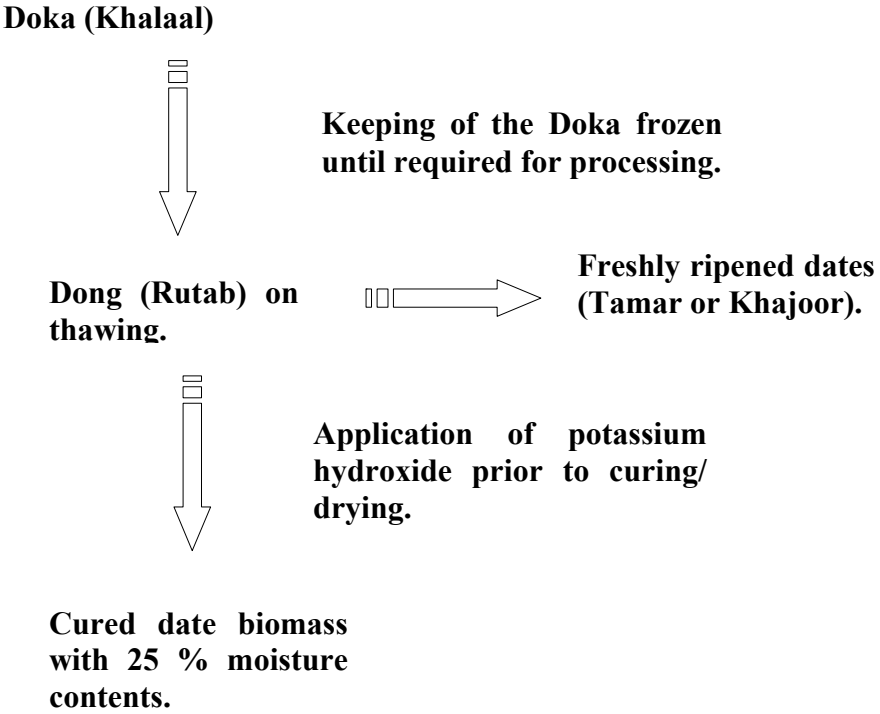

Fig. 1. Proposed scheme to streamline production of 'Dhakki' dates so as to make fresh dates available the year round.

Influence of Microwave Radiation on Ripening of 'Dhakki' Dates

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Keywords: 'Dhakki' dates, *Phoenix dactylifera*, microwave radiation, ripening, quality, maturity, Pakistan

Abstract

Research was conducted to investigate the impact of microwave (Mw) radiation for stimulating the ripening of 'Dhakki' date dates. The date fruits picked at four sub-khalal (doka) maturity stages well below the on-set of ripening, were radiated for 10 to 50 s at three microwave power levels of low (210 W), low medium (360 W) and medium (480 W) intensity. The radiated samples along with non-radiated control were placed for ripening/curing at 40°C under fluorescent light, dark or sun. The effectiveness of a treatment was evaluated by estimating ripeness (%) after 24, 48, 120 and 144 h incubation. Ripening/curing of the dates increased gradually during the incubation displaying no difference in the rate whether incubated under dark or light. However, the Mw radiation boosted up the date forming process seven times, reducing the curing period sharply from 288 h for the control samples to 40 h for the samples that were Mw radiated for 50 s at 480 W. The stage of maturity of the dates also offered a positive response to the ripening process thereby enhancing yield of the product substantially, and the maturity level at the late doka stage with hardness level of 180-200 mm Hg cm^{-2} believed the attractive maturity range for the ripening. Moreover, the radiation at 480 W facilitated early harvesting of dates prior to attaining optimum maturity stage thereby saving at least two weeks hanging period of the fruits on the tree, and hence the damage by monsoon rain is expected to be reduced whilst the desirable qualities of the dates held intact. However, exposing the dates beyond 480 W manifested heat roasting and caused an undesirable baked flavour.

INTRODUCTION

The 'Dhakki' cultivar of dates (*Phoenix dactylifera* L.) is well-known in Pakistan by virtue of its high quality. However, it is a late cultivar and very much sensitive to moisture. In the month of August, when the fruit is 26-27 weeks old it attains Khalal or locally called "doka" stage. At this stage the fruit is fully mature and has attained its full size and turns its colour from green to yellow. 'Dhakki' fruit at the doka stage is astringent, relatively hard, unpalatable and unsuitable to pick for ripening/curing purposes. Later on the fruit acquires the stage of rutab locally called as "dong". The dong stage is characterized by the appearance of a translucent brown colour near the tip of the fruit, and the fruit gains commercial maturity. At this stage the fruit becomes relatively soft and bitter tannins precipitate down, and the fruit is ready for picking from the tree for the ripening/curing process. Unfortunately this is a period when monsoons are well inside of Pakistan. Hence the 'Dhakki' date which is experiencing ripening suffers heavy post harvest losses and quality degradation on account of severe weather conditions of high humidity at relatively high summer temperatures. This adverse situation arises on account of monsoon rain fall that persists for weeks.

Mostly the traditional methods are used for ripening/curing by which the dates on mats are exposed to sun at open air. Due to persisting raining conditions a sizeable amount of the product turns out to be moldy, fermented and dusty. In order to minimize post harvest damages and improve quality it becomes imperative to take scientific measures. It is worth to consider picking of the dates when they are still hard almost at

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doka stage and get them processed preferably before the fall of monsoon season.

Microwaves received considerable attention in the food industry as early as 1947. Microwaves have been used for packaging of bread (Cathcart et al., 1947), rapid cooking and blanching of foods (Proctor and Goldblith, 1948), blanching of pasta (Maurer et al., 1971), production of bakery materials (Stein, 1972; Lorenz et al., 1973), popping of popcorn (Lin and Anantheswaran, 1988), drying of bananas (Garcia et al., 1988), and comparative study of microwave with conventional methods (Huxsoll et al., 1970a,b; Purcell and Walter, 1988). The use of microwaves for assessing fruit maturity has also been reported (Lee, 1998).

Importance given to microwave radiation is due to its rapid heating rate with more penetration capacity especially in high moisture range, whereas at least 4700°F units of heat energy are required to accelerate the fruit ripening process of date cultivars (Chandler, 1958). This led to examining the impact of microwave radiation on the ripening of 'Dhakki' date, keeping in mind that Mw are a high source of heat energy that may enhance the ripening process which requires heat energy. Recently Saleem et al. (2002) reported a positive response of microwave radiation on the ripening of 'Dhakki' dates. They reported that Mw radiation of 210, 360 and 480 W energy improved ripe-fruit size, appearance and juiciness. Hence the objective of this study is to explore the potential use of microwave energy for accelerated ripening of the dates, so as to complete the ripening process prior to fall of seasonal monsoon rain and to reduce the impact of the rain damage.

MATERIALS AND METHODS

'Dhakki' date fruits at various doka stages were procured from orchards belonging to progressive growers situated in the vicinity of Dera Ismail Khan, and brought to the Department of Food Science and Technology, Faculty of Agriculture, Gomal University Dera Ismail Khan for investigation. The reported data are based on two years observations. Although three local date cultivars i.e., 'Dhakki', 'Basra' and 'Shakri' were investigated for the influence of microwave radiation on ripening, however, most of the results reported in this study are related to the 'Dhakki' cultivar.

Healthy dates at mature khalal (doka) stage without having signs of infestation and deformity were picked manually from trees, well washed under tap water containing surf detergent to reduce microbial load, dust, dirt or any adherent spray chemicals. These were then dipped in 0.1% aqueous solution of sodium benzoate, blotted on paper wiping off excessive water.

About 5 kg equal sized dates were taken for each set of treatments and divided into 30 equal size lots comprised of 10 dates each and taken in petri glass dishes. The samples were placed separately in a microwave oven for the radiation. The microwave oven was of "National" NN-6206 brand, and Matsushita Electric Industrial Co. Ltd. Japan supplied it. The oven was equipped with input of 1200 W, 5.6 A, 220 V, 50 Hz cycle and output maximum energy of 600 W, 2450 MHz at full power. It had a variable power control varying from 80 to 600 W. The oven had a 30 min timer with defrost function and "Magic-Platter" automatic turntable facilities. The time of exposing the samples to radiation was however controlled manually by shutting the oven off after a desired time. Three radiation levels of low (210 W), low medium (360 W) and medium (480 W) intensities are examined. The samples were radiated for l0, 20, 30, 40 or 50 s at a required radiation level. These were then placed for ripening/curing inside a fluorescent light of a 40 W fitted incubator, or kept under dark or sun and incubated for different periods varying from 24 to 144 h at 40°C. The ripening percentage was measured after 24, 48, 120 and 144 h curing period. The experiments were conducted in duplicate and the average value was recorded. Whenever required control samples were run side by side to compare the results.

In order to find out the effect of the stage of maturity on the ripening of 'Dhakki' dates the dates were harvested weekly. The picking of dates was done manually from the tree in August. The samples were radiated for 50 s at the required Mw power and then

placed for curing in the incubator operating at 40°C for 48 h under fluorescent light. The level of maturity and ripening status of the samples were assessed by means of a hardness-testing device developed in the laboratory (Saleem et al., 2002). The device worked on the principle of Aneroid Sphygmomanometer. It was comprised of a glass barrel with piston inside, a stainless steel bar having 1 mm penetrating surface area, and a rubber blow ball attached to a pressure gauge. The barrel was attached to the pressure gauge while the piston was mounted with the stainless steel bar. The barrel was fixed vertically on a stand in such a way that the needle just touches the fruit surface. The fruit required for hardness testing was placed on the base under the tip of the pressure needle. Air pressure on the piston was applied increasing the pressure gauge gradually. Maximum pressure was noted as soon as the needle punctured the fruit surface. At least 5 readings were noted on each fruit taking randomly altogether 10 dates from the lot required for maturity evaluation, and average pressure reading (mm Hg cm^{-2}) was recorded. Graduate students of the department assessed flavour and taste of the date according to Jellinek (1985).

Estimation of Ripeness

The ripeness defined as the change in color of fruit from yellow to brown was assessed visually and the ripened surface area measured with the help of transparent paper scale. The ratio of the ripe portion to total fruit surface was calculated (R). A fruit with ≥75% ripe portion was considered as fully ripe and fully cured. The overall ripening of a sample was computed applying the following empirical formula. The estimated ripening (%) virtually reflects the product yield.

Estimated ripening (%)=(∑[ripeness ratio per fruit (R) × number of dates] × 100)/total number of dates

RESULTS AND DISCUSSION

Microwave Radiation Studies

The 'Dhakki', 'Basra' and 'Shakri' date cultivars were harvested at their appropriate stage and treated with the desired Mw radiation intensities by exposing them for various periods and then incubated for ripening/curing.

Incubation and Ripening

The ripening of dates increased gradually with increasing period of incubation regardless of whether the dates were incubated under fluorescent fight, in the dark, or in the sun (Figs. 1-3). However, the rate of ripening was slightly faster in the control samples that were incubated in the sun. The sun-ripened dates were more acceptable for their appealing bright colour. It was further observed that the rate of ripening increased sharply beyond 120 h curing period giving a noticeable ripening difference in dates incubated for 48 or 120 h or more.

Radiation and Ripening

Regardless the intensity of Mw radiation, the rate of ripening increased with the time of exposure from 10 to 50 s. Comparing selected dates incubated for 120 h in the light, microwave radiation for 40 to 50 s at any intensity increased ripening rapidly (Figs. 1-3). The efficiency was further enhanced on increasing radiation from zero level to low (210 W), low medium (360 W) and to medium (480 W) intensities by at least 2.2, 2.7 and 7 times, respectively. A radiation intensity of 480 W resulted in a substantial reduction in the curing period and demonstrated the single major factor responsible for rapid ripening (Figs. 4 and 5). 'Dhakki' dates exposed to microwave radiation with medium intensity of 480 W took only 48 h to achieve 75% ripening, and dates exposed to microwave radiation at low and low medium intensities took 130 and 108 h, respectively, whereas the control dates required at least 288 h to reach the equivalent level of ripening (Fig. 4). The incubation time necessary to achieve 75% ripening was estimated from the

plot of the ripening percentage and period of incubation under light, dark or sun.

The radiated fruits after curing were larger in size than those not exposed to microwave radiation, confirming previous observations (Saleem et al., 2002). Dates attained such peculiar characteristics due to fast and deep heating by microwave radiation. Gaseous vapors inside the dates during microwave radiation stood against the collapse of the dates. However, medium high intensities (540 W) or greater were injurious to date fruits, and the radiated dates exhibited a baked flavour and were injured oozing liquid out as a result of excessive microwave heating. Purcell and Walter (1988) compared carbohydrates of sweet potatoes baked by convectional heating with that of microwave heating. They reported that the sweet potatoes baked in the microwave contained less reducing sugars and dextrins and more starch than those cooked in the convection oven. In the present study the dates cured after microwave treatments tasted less sweet compared to the control dates. Since the biochemical reactions associated with the maturation/ripening process are liable to recede at least partially as a result of denaturation of the metabolic enzymes by microwaves heating, it is likely that the microwave radiation retarded enzymic conversion of high molecular carbohydrates to sweeter mono- and di-saccharides. Because the microwave-ripened dates are less sweet than dates cured under conventional means, the radiated product may be more acceptable.

Maturity and Ripening

The stage of maturity of harvested dates was a major controlling factor affecting the curing of dates (Fig. 5). Dates harvested with maturity well below the minimum required level exceeded a hardness level of about 300 mm Hg cm^{-2}. The ripening and curing was unsatisfactory, and resulted in low yield. However, microwave radiation improved the ripening to a considerable extent provided the harvesting was within the minimum required stage of maturity below the 250 index. The dates picked during the first week of August and exposed to medium level microwave radiation exhibited ripening equivalent to the control dates harvested as late as the fourth week of August. Microwave radiation thus allows the dates to be harvested at relatively less maturity, and offers considerable provision to avoid the rainy period and improve yield of acceptable dates. However, dates given no microwave radiation and harvested prior to doka stage appeared furrowed and smelled hay-like after incubation. While dates exposed to microwave radiation looked and tasted like chhohara instead of ripened dates, chhohara being a local description of dried dates made after cooking the hard mature whole dates harvested at the khalal stage in boiling water.

Regardless as to whether the dates were radiated, the harvesting of dates after the 2^{nd} week of August furnished highly promising results. The yields in terms of percent ripening of the dates were substantially increased. The effect was even further improved when the dates were also exposed to microwave radiation, and still greater on increasing the intensity of the radiation to medium level. The period of ripening/curing of the dates was reduced to 48 h. However, on increasing the microwave radiation energy further to 540 W, an adverse effect was exhibited and the radiated dates appeared baked and wounded, oozing liquid as a result of overheating.

'Basra' and 'Shakri' Cultivars

The effect of microwave radiation on the ripening of 'Basra' and 'Shakri' date cultivars was also investigated. Both cultivars responded well to the radiation at low, low medium and medium energies, and the effectiveness of the radiation regarding ripening percentages for these cultivars was greater as compared to 'Dhakki' (Fig. 6). Maximum response was displayed by 'Basra' that became fully ripe and fully cured within 48 h by receiving radiation at the level of low medium intensity for 50 s. Whereas minimum response to microwave radiation was exhibited by 'Dhakki', giving only 29% ripening at the equivalent radiation level. The variable response given by different cultivars might be due to difference in date size and skin thickness. 'Dhakki' exhibits the thickest skin among the selected date cultivars and tolerated exposure to higher intensities of

microwave radiation.

CONCLUSIONS

There was a great positive impact of microwave radiation on the ripening of the dates. The curing period was proportionately shortened on the appropriate use of microwave radiation, and allowed early harvesting of dates by 2 weeks. By applying microwave radiation, substantial losses caused by monsoon rain were largely avoided. The microwave radiated dates attained superior sensory quality. However, radiation exceeding 480 W energy exhibited harmful effects on date quality, and over-heated dates manifested a baked or cooked flavour. Further, such dates acquired soft textures with some liquids oozing out of the dates inviting microbial contamination and growth. Nevertheless, microwave radiation to a medium level of 480 W accelerated the ripening of 'Dhakki', 'Basra' and 'Shakri' dates and shortened the ripening period substantially. The radiated dates retained their original bigger size and appearance, while the shape of the dates became distorted on ripening by the conventional technique. Keeping the treated dates under the sun further improved the color of the dates. Since the dates became soft as a result of microwave radiation, they acquired an acceptable ripening level even if harvested earlier at lower level of maturity; thereby increasing the overall yield of the acceptable cured dates. Based on the present study, microwave radiation may have great potential to improve the ripening of date cultivars. This is the first study on exposing harvested dates to microwave radiation, and the technique may conceivably have great prospects for commercial application. Although there may be some disadvantages in exposing dates to microwave radiation (Kopp, 1998), the radiation technique offers increases in yield and substantial savings in time, floor space, cleaning and maintenance.

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Figures

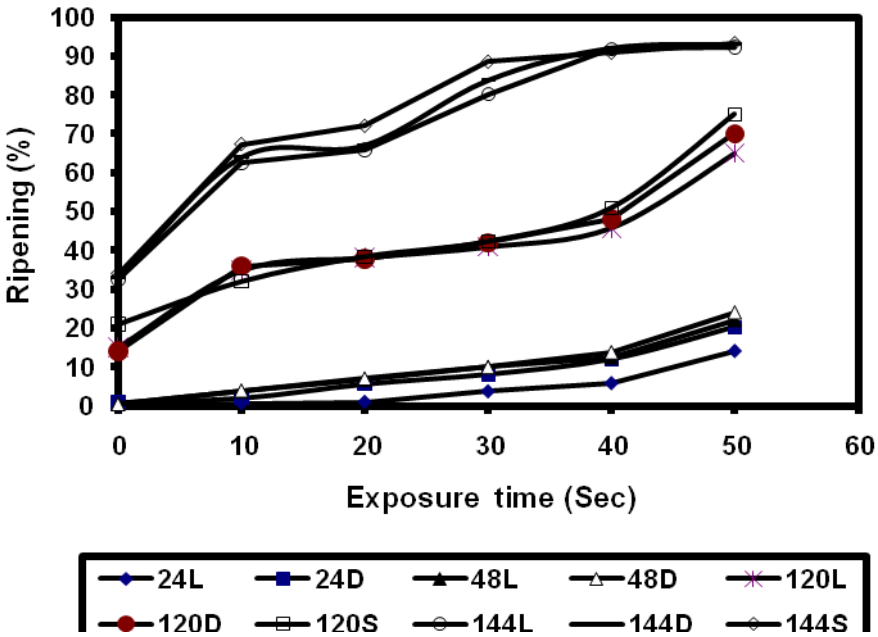

Fig. 1. Effect of Mw exposure time at low intensity (210 W) radiations on ripening of 'Dhakki' dates cured under light, dark and sun at 40°C.

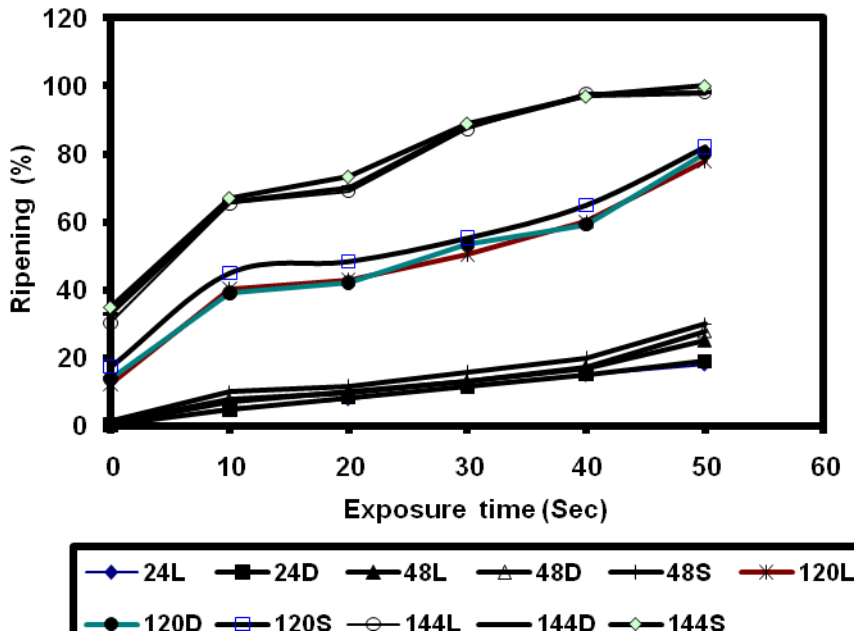

Fig. 2. Effect of Mw exposure time at low medium intensity (360 W) radiations on ripening of 'Dhakki' dates cured under light (L), dark (D) and sun (S) at 40°C.

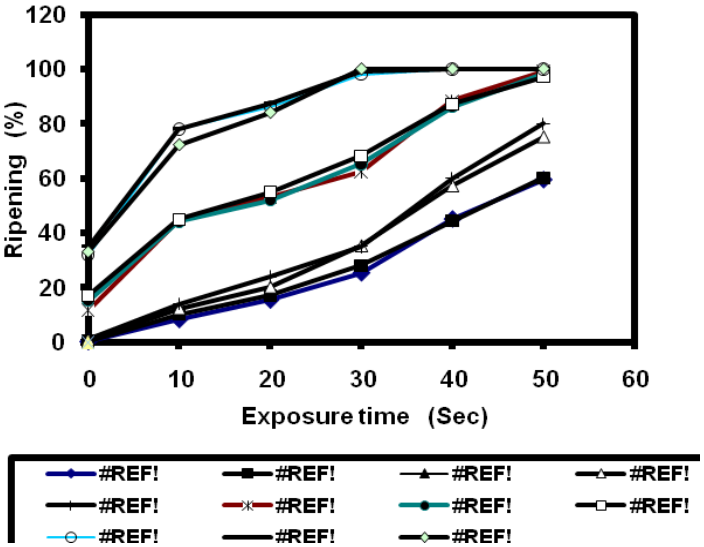

Fig. 3. Effect of Mw exposure time at medium intensity (480 W) of radiation on ripening of 'Dhakki' dates cured under light (L), dark (D) and sun (S) at 40°C.

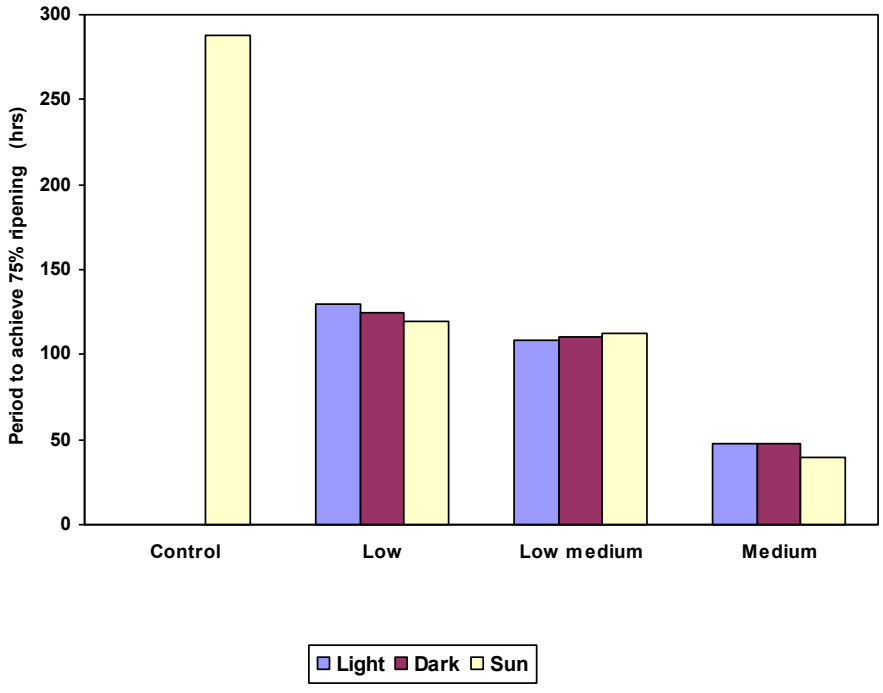

Fig. 4. Effect of level of Mw radiations on the period for 75% ripening of 'Dhakki' dates cured under light, dark and sun at 40°C.

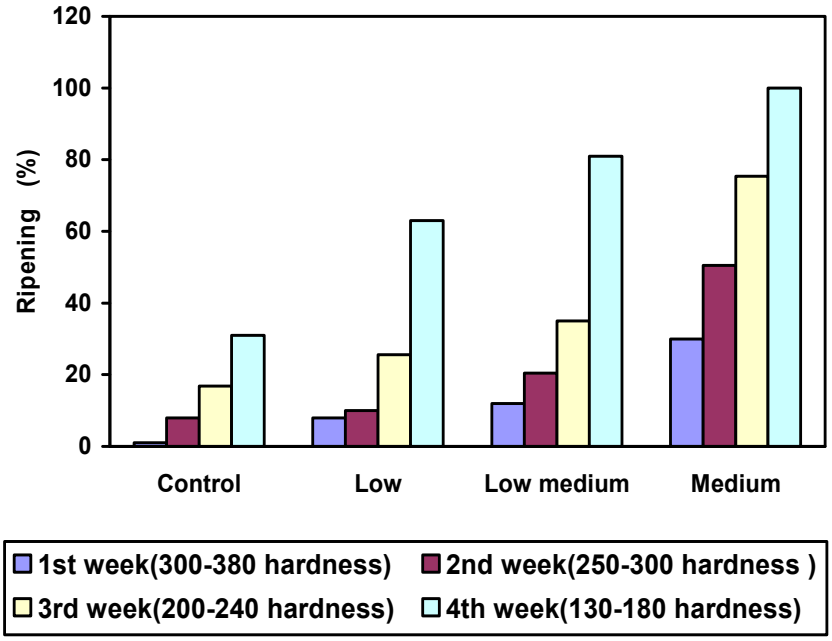

Fig. 5. Effect of maturity levels on ripening of 'Dhakki' dates Mw radiated for 50 s and incubated for 48 h at 40°C.

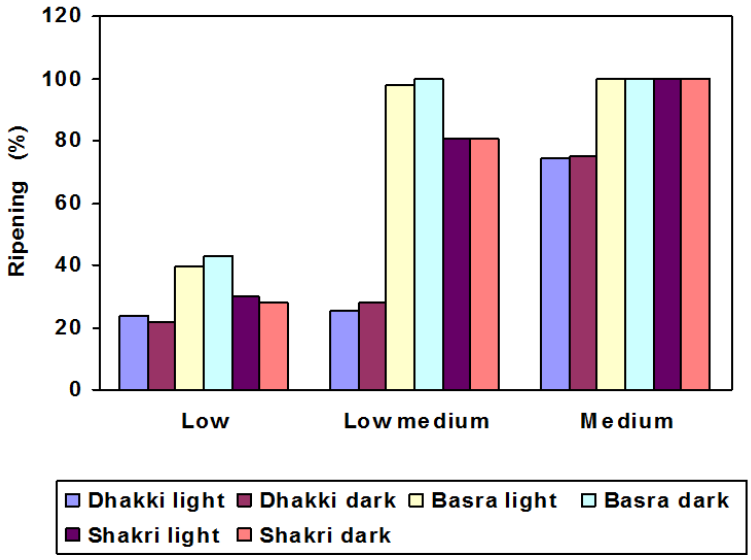

Fig. 6. Effect of intensity levels on ripening of 'Dhakki', 'Basra' and 'Shakri' date cultivars Mw radiated for 50 s and cured for 48 h at 40°C.

Impact of Artificial Ripening to Improve Quality and Yield for the Export of 'Dhakki' Dates

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Abstract

The most promising and locally developed 'Dhakki' date cultivar (*Phoenix dactylifera* L.) of Dera Ismail Khan, is considered amongst the few world-leading cultivars. Small seeded mature fruit has a substantially large size (5-6 cm long and 2-3 cm thick) and heavy weights (20-25 g/fruit). Though astringent at doka (khalal) stage, it develops a fine texture and relish taste on ripening, and fetches a high price on the market. 'Dhakki', a crop of national importance is facing diversified problems. Coincidence of ripening period with the stormy monsoon season is the most damaging factor for 'Dhakki', which is a late cultivar suffering quality degradation and post harvest losses of enormous amounts. Further, the product becomes awfully contaminated and fermented using traditional techniques of exposing the fruits on mats to the sun at open air. The aim of the study was to induce a well advanced rapid artificial ripening in 'Dhakki' fruits harvested at firm and astringent doka stage, and complete curing/drying before the fall of monsoon. Brine and vinegar acid has been investigated as ripening initiator/accelerator, applied individually and/or in combined form at 0.25 to 3.5% concentration. The doka immersed in a treatment solution for 5 min was ripened/cured in an aerated incubator at 38 to 40°C for 72 h. Observing changes in color shade, fruit weight, pulp, texture, total soluble solids, appearance and the extent of ripening assessed the efficiency of the treatment. All of the treatments induced ripening of varying degree, however, 2% brine appeared highly effective for inducing accelerated ripening. The process caused 75% excessive yield with premium quality product and saved 2-3 weeks period required for attaining the dong (rutab) stage, besides overcoming the expected excessive damages caused by monsoon and insect/bird attack.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is well recognized ever since the birth of the human race and since then it has been playing an important role. It nourishes millions all over the world and contributes significantly towards development and prosperity especially to those living in the Arabic countries. The Prophet Muhammad (PBUH) encouraged his followers to honor the tree by calling it their "Aunt". Muslims consider it as a virtue to distribute and eat dates particularly at the occasion of 'Iftar' in the month of 'Ramadan'. Date fruits being sweet and palatable, are consumed as a staple diet, and provide 2500-3000 calories/kg of physiological energy, a high amount of potassium, easily digestible carbohydrates, and a significant amount of cellulose and hemi-cellulose materials, and fortunately the fruit is practically free from sodium and cholesterol elements. On the basis of composition and usefulness the dates are considered a divine gift particularly for heart patients. The dates are one of our important cash crops, and a good source of foreign exchange earnings. Pakistan is the 5th largest date producing country in the world constituting about 11% of total world production (Anon., 2008). 'Dhakki' date of Dera Ismail Khan is the most promising local cultivar reckoned amongst the top few world-leading cultivars. The date is quite popular for its large size (5-6 cm

long and 2-3 cm thick) and fruit weight (20-25 g/fruit) with a fine texture and relish taste (Baloch, 1999) fetching a high price on the market.

Although the 'Dhakki' date is a crop of national importance it faces diversified problems. Being a late cultivar its harvesting period coincides with the high humid and stormy monsoon season and the date consequently does not get a chance to ripen properly, and insect/bird pest problems and physical damages are common. Moreover, a large quantity of freshly ripened fruits available from early date cultivars glut the market and the limited available provision with the farmers is preoccupied, consequently the 'Dhakki' date suffers heavy crop losses and quality degradation. Quality of the dates is further impaired using traditional processing techniques of exposing the fruits on mats to the sun at open air. Besides a sizeable amount of the product turns out as dusty and harmfully contaminated as well as fermented due to insufficient drying and storage facilities. In order to improve quality, minimize post harvest damages and reduce financial pinching losses it becomes imperative to ripen the dates before the fall of this critical season. Information regarding ripening of dates using artificial means is inadequate, and the few reports available are cultivar specific (Kalra et al., 1977; Asif and Al Taher, 1983). In our previous study we have reported ripening/curing of 'Dhakki' dates using microwave radiation (Saleem et al., 2002; Baloch et al., 2003). The present study is therefore aimed at investigating the impact of table salt and vinegar acid as ripening promoters for 'Dhakki' dates.

MATERIALS AND METHODS

The 'Dhakki' fruits at mature hard doka stage (250-300 hardness, Baloch et al., 2003) were procured from the Agriculture Research Farm, Ratta Kulachi, Dera Ismail Khan and harvesting of the fruits was completed before the onset of the monsoon season. Healthy and non-infected fruits were selected for the experimentation. The fruits were given cleaning and washing before subjecting to ripening treatments with solutions of brine (0.25 to 3.5%) and vinegar acid (0.25 to 2.5%, Table 1). The fruits were divided into 13 lots each of equal size (1 kg) and maturity. The samples were immersed in the respective solution (1 L) for 4-5 min at room temperature, allowed to drain and spread out separately on stainless steel trays. The samples were incubated for 72 h in an aerated incubator at 38 to 40°C. A control with water dip treatment was run simultaneously to compare the effectiveness of the ripening stimulants.

Data Collection

Ripening parameters were evaluated immediately after harvesting and ripening treatments. Performance of a stimulant whether used singly or in combination was judged by following changes in color, texture, average fruit weight, percent pulp and total soluble solids (TSS). The color of the fruit was recorded by visual observations comparing with a horticultural color chart (Kader, 1992). Average weight of fruit pulp was calculated by taking weight of the ripened seed-free fruits and weight recorded. The texture hardness of the ripened fruits was determined by a hardness testing device that was previously developed in our laboratory (Fig. 1, Baloch et al., 2003). The degree of ripeness (which also represents yield) and the total soluble solids were estimated according to the methods reported earlier (Saleem et al., 2002; Baloch et al., 2003). An organoleptic test for fruit appearance was performed according to Jellinek (1985). For the evaluation, the samples were distributed to 10 panel members from the trained graduate students of the Department of Food Technology. The evaluation was based on a hedonic scale ranking from 1 to 10 where 1 ranks for very poor, and the 10 stands for the best sample. The samples were evaluated twice and the average values reported.

RESULTS AND DISCUSSION

There are at least 4 stages of development and ripening for dates. These include kimri, khalal, rutab and tamar (Fig. 2). The fruits at khalal being fully-grown and mature tend to ripen to acquire half-ripened rutab stage, which then leads to fully ripened tamar

form of reduced moisture contents. The changeover of stages through normal ripening process while the fruits are attached to the tree requires a 5-6 weeks period in addition to 2-3 weeks for curing/drying and to reduce biomass moisture necessary to give shelf stability to the product. The idea behind the study was to artificially induce speedy ripening in fruits of firm, astringent and yellow profile at doka stage using stimulants, and to complete curing/drying rapidly cutting down most of the period normally required for rutab and tamar conversion processes (Fig. 2). Adapting the proposed methodology it is highly likely to avoid devastating damages caused by monsoon and other factors. The results are discussed in the following sections.

Changes in Fruit Color

Color of fruit is an important index, which plays a pivotal role in the marketing value and quality determination. Similarly, variation in the color is closely associated with the extent of ripening. However, different cultivars display their own color pattern on ripening. 'Dhakki' dokas change their color giving different shades from yellow to brown during ripening/curing. The color tends to change as a result of biochemical activities as induced inside the doka by the chemical stimulants. The range of colors is presented in Table 2.

All of the treatments exerted a positive effect on this important property. The 'Dhakki' date while undergoing ripening, changes color from light yellow at khalal to golden brown at the rutab stage. However, the color shade varied with the nature of the treatment applied and the extent of ripening (Table 2). The fruits while ripening under the influence of the chemicals changed color much earlier compared to the control treatment. The effect was highly pronounced with brine treatments and that no matter whether the salt was employed alone or in combination with vinegar acid, gave an acceptable ripening color. The data further revealed that brine at 2% concentration in conjunction with the vinegar acid at any ratio imparted a desired reddish brown and attractive color with good eye appeal. However, 2% brine treatment applied singly offered superior color shine surpassing all other treatments, whereas the color produced by vinegar acid, as a single treatment was least acceptable. The results indicate however, that both of the chemicals triggered physiological changes activating ripening enzymes. Brine responded swiftly in bringing forth changes in desired color on ripening. Asif and Al Taher (1983) had also reported a similar finding for 'Khasab' dates, however the same reagents reportedly appeared ineffective for 'Zaidi' and 'Thoory' date cultivars (Kalra et al., 1977).

Fruit Texture

The effect of stimulants on the texture of date fruit is given in Table 3. The texture of the fruits treated with the control treatment was negligibly soft and firm, whereas brine applied singly as well as in combination with vinegar acid conferred a soft texture to the fruits. Further, the samples treated with T_3, T_4, T_5, T_{10}, T_{11}, T_{12} and T_{13} were found to be much softer, more pulpy and juicy. It is also noted that the fruits cured by these treatments were more flaccid at the surface; however increased firmness was noticed towards the center of the fruits. This indicates that the process of ripening initiated from the outer surface of the fruits, and progressed inside to the fruit core. The fruits ripened by the control treatment showed a uniform softness throughout, though it was to a lesser degree. Previously the microwave radiation had also caused a uniform ripening throughout the fruit body (Baloch et al., 2003). Kalra et al. (1977) also reported that salt and vinegar acid produced surface ripening of the dates. Further, wrinkling was highly pronounced in the case of fruits ripened using the control treatment; however, fruits treated with salt were least furrowed. Wrinkling of the fruits most probably resulted from collapsing of the weakened tissues with disproportionate moisture losses from the fruits undergoing ripening.

Fruit Weight and Pulp Contents

Data on average fruit weight and pulp contents are presented in Table 4. Salt

treated fruits gave higher fruit weight and pulp contents than those for water (control) or vinegar acid treated samples. A slight increase in fruit weight or pulp was seen in the case of combination treatments, and the amount was further increased on increasing the concentration of vinegar acid or salt. However, the extent of rise was much pronounced in case of salt treatment. Nevertheless both of the treatments performed better than the control. The higher weight for the brine treated fruits may be attributed to high pulp percent and to more juiciness. This finding is dissimilar to the results from 'Khasab' date where weight and pulp percentage was lower in fruits treated with vinegar acid alone or in combination with salt as compared to the control samples (Asif and Al Taher, 1983). Slight variation in the results might have been caused by cultivar related or procedural factors.

Total Soluble Solids (TSS)

Data regarding total soluble solids are presented in Table 4. The data show that the samples with treatment T_4 and T_5 had the highest amount of total soluble solids compared to other treatments, while the effectiveness of treatment T_8 and T_9 is of a minimum degree. TSS is considered in relation to total weight of the fruit. It is generally specified as to what extent the TSS is present in relation to water. It means that the freshly harvested dates are said to contain more water and less total soluble solids. It shows that a decrease in moisture or water contents results in more concentrated fruits TSS. Further, water evaporates during incubation for ripening/curing and moreover, the insoluble fruit materials get solublized simultaneously as a result of breaking down of polymers through ripening. This is what has presently been observed from the data (Table 4). It is however, indicated clearly that treatment with salt singly or in combination with vinegar acid has responded well giving a higher percentage of TSS. The rise in TSS from brine treatment resulted from conversion of water-insoluble protopectin to pectin on stimulation of the ripening process by the salt. Moreover, the higher amount of the soluble solids in salt treated samples prevented moisture from evaporating easily, hence the cured fruits maintained their juiciness. Fruit treated with vinegar acid singly showed a lower percentage of TSS as against the control. This is probably due to the greater amount of water loss from the control samples. The present findings showed a similarity with earlier reports (Asif and Al Taher, 1983; Cheema, 1986; Ali, 1989; Thatai and Kalra, 1982). However, some of the berries after treatment with 3.5% brine and those from combination treatments became over soft and developed a disagreeable taste and flavor.

Fruits Ripening

Apparently all of the treatments helped inducing ripening of 'Dhakki' date to a certain degree (Fig. 3). However, the extent of ripening varied with the nature of the stimulant and amount of the stimulant for application. Increasing the concentration of brine resulted in a progressive increase in the percentage of fruit ripeness and color change. Vinegar acid also showed a similar trend, but a far lesser degree. Combination treatments also induced a considerable amount of ripening. Maximum ripening as high as 90% was observed for 2% brine containing 2.5% vinegar acid treatment, followed by 88 and 87% for 2% brine with 1.5% vinegar acid and 3.5% brine treatments respectively. Only a few fruits from the control treatment approached the required ripening level, thus displaying very poor performance. The findings agree with the results of Kalra et al. (1977) who reported that the increase in salt concentration progressively increased the dong formation of 'Khadrawi' and 'Shamran' date caltivars, and addition of vinegar acid enhanced the effect, but vinegar acid alone was ineffective. They further reported that none of the treatments induced softening in 'Zaidi' and 'Thoory' cultivars. In our study however, the effect of vinegar acid alone on 'Dhakki' date was not significant, though more effective compared to the control treatment. Currently, brine at 2% concentration has accelerated the ripening process efficiently and yielded an optimum quality product. The detection of invertase, polygalacturonase, cellulase, polyphenol oxidase and other ripening enzymes has been reported in dates at the doka stage (Hasegawa and Smolensky,

1971; Hasegawa and Maier, 1980). It has further been reported that polygalacturonase and cellulase were absent or present at only a low level in the green date fruit but displayed large increases in activity during ripening (Hasegawa and Maier, 1980; Saleem et al., 2002).

Ranking Values/Organoleptic Ratings

The brine treated fruits appeared superior in ranking to those from the vinegar acid (Fig. 4). Treatment with vinegar acid caused light to dark brown unappealing spots on some of the fruits. The effect was intensified with increasing concentration of the acid. Combined treatments containing acid also affected the appearance, but to a lesser degree. Some of the fruits from the 3.5% brine treatment and those from combination treatments with 2% acid appeared over soft. Moreover, some of the acid treated fruits showed signs of fungal growth on the surface. Fruits treated with the highest concentration of brine (3.5%) were slightly salty in taste while an acid-taste was not detectable in acid treated fruits, except for those over softened fruits, which showed a slightly acidic flavor. The softened fruits were not astringents in taste and flavor, and the tannins of the fruits are likely to have been precipitated.

CONCLUSION

It is shown that brine and vinegar acid exert a positive response inducing the ripening in 'Dhakki' dates and accelerated the pace of ripening/curing. However, brine used alone proved more effective and the quality changes in terms of color, taste and appearance were more pleasant. Previously it was demonstrated that the ripening of doka at 250 hardness units is triggered by application of an optimum dose of microwave radiation (Baloch, 1999; Saleem et al., 2002; Baloch et al., 2003). Since at present, considerable ripening also occurs in doka with 250 hardness consequent upon the ripening activators, it proves beyond doubt that leaving the fruit on the tree for want of dong formation through natural process, is quite unjustified. It is highly likely that the post harvest processing can be completed at least 2 weeks before the start of the monsoon season, hence avoiding adverse consequences, provided the doka with full maturity is treated with the optimum dose of reagents, and allowed to cure under appropriate conditions. It is postulated that sufficient amount of the ripening enzyme existed as an immobile form in unripe yet fully mature fruits. The ripening agents possibly disrupt the epidermal cell and the protoplasm thereby releasing and activating the enzymes. Ripening by involvement of enzymes like invertase, polygalacturonase, cellulase, pectin estrase and polyphenol oxidases, etc., causes the structural parts like pectin and cellulose that hold cells together to became solubilized, and the tannin precipitated. As a result the fruit manifests the ripeness states of rutab and tamar precipitating out tannins, tasting sweeter, softening in structure and introducing changes in fruit color and other ripening associated quality parameters. The extent of modification varies at a rate depending upon the stage of fruit maturity and the environmental factors responsible for the ripening/curing of the fruits. The present findings agree with our previous views that there is no point in waiting till dong formation on-tree once the fruit attains full maturity of doka level. Although treatment with salt involves some extra work and cost, however, the grower can resort to remedies to save his perishable commodity at a khalal stage. The harvesting period can easily be shortened by the manipulation of enzyme inducing techniques thus saving the fruits from significant damage. Addition of enzymic preparations to dates has been reported to hasten desirable conversions. Various enzymic preparations containing invertase (Smolensky et al., 1976; Elzoghbi, 1994) for texture and appearance of "sugar wall" dates, and pectic enzymes and cellulases (Smolensky et al., 1973, 1975) for quality improvement of mixed green and substandard dates have been reported. The ripening and curing processes are simultaneously initiated as a result of chemical applications transforming the perishable khalal of 'Dhakki' fruit to its preserved tamar form, and hence bypassing the on-tree rutab phase successfully. The conversion resulted in a decrease in fruit weight due to moisture loss, increase in soluble solids, yielding a soft pliable texture, a browning of the mass, and the development of the taste and flavor of

tamar date. In the present study the treatments possibly activated ripening enzymes which brought changes resulting in fruit softness, color changes from yellow to reddish brown and taste development. It is further concluded that the process of ripening is initiated from the outer surface of the fruit and progressed inwards.

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Tables

Table 1. Symbols for the brine and vinegar acid stimulants.

Symbols	Stimulants
T_1	Control (dipping in distilled water)
T_2	0.25% brine solution
T_3	1.5% brine solution
T_4	2.0% brine solution
T_5	3.5% brine solution
T_6	0.25% vinegar acid
T_7	0.5% vinegar acid
T_8	1.5% vinegar acid
T_9	2.5% vinegar acid
T_{10}	Solution containing 2.0% brine + 0.25% vinegar acid
T_{11}	Solution containing 2.0% brine + 0.5% vinegar acid
T_{12}	Solution containing 2.0% brine + 1.5% vinegar acid
T_{13}	Solution containing 2.0% brine + 2.5% vinegar acid.

Table 2. Effect of stimulants on color of 'Dhakki' doka.

Stimulants	Color of the ripened/cured fruits
T_1	Slightly brownish but not shining
T_2	Amber and attractive color
T_3	Shining brownish color developed
T_4	Reddish bright brown color with much attraction
T_5	Translucent reddish brown color
T_6	Light brown but not attractive
T_7	Dark brownish color
T_8	Dirty brownish color
T_9	Brown and not attractive
T_{10}	Darkish brown and not attractive
T_{11}	Darkish brown and acceptable
T_{12}	Darkish brownish and satisfactory
T_{13}	Darkish brownish with little shinning

Table 3. Effect of stimulants on the texture of 'Dhakki' doka.

Treatments	Texture of ripened/cured fruits
T_1	Firm and least soft
T_2	Less soft texture without stickiness
T_3	Soft textured and pulpy
T_4	Soft texture, pliable, pulpy and smooth surface
T_5	Very soft and slightly sticky surface
T_6	Compact and sticky surface
T_7	Soft textured and pulpy
T_8	Pulpy, soft and sticky
T_9	Very soft, loose and sticky
T_{10}	Soft and juicy
T_{11}	Soft, juicy and sticky
T_{12}	Soft, loose and sticky
T_{13}	Very soft and over juicy

Table 4. Effect of brine, and vinegar acid applied singly and in combination on weight, pulp and total soluble solids of 'Dhakki' doka.

Treatments	Wt. of fruit (g)	Pulp (%)	TSS (%)
T_1	13.0	95.4	67.2
T_2	15.4	95.8	68.8
T_3	15.6	95.7	69.2
T_4	16.2	95.1	70.0
T_5	16.3	95.6	70.4
T_6	13.1	94.6	65.6
T_7	13.5	94.4	66.4
T_8	13.6	94.2	64.8
T_9	13.8	93.8	64.0
T_{10}	16.6	95.3	67.2
T_{11}	16.8	95.1	66.8
T_{12}	16.8	95.8	66.0
T_{13}	17.2	95.9	65.6

Figures

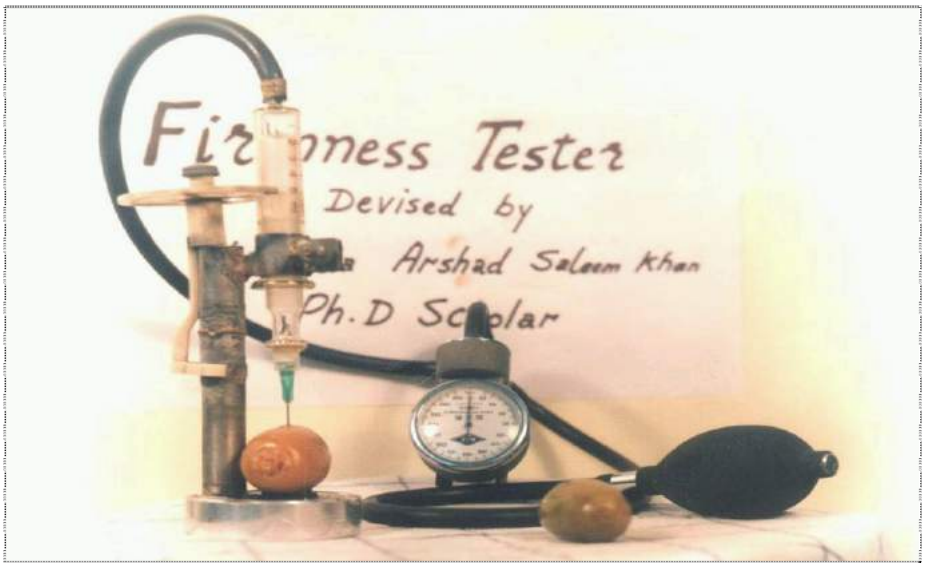

Fig. 1. Hardness testing device for 'Dhakki' dates developed by the author (Dr. Shahzada A. Saleem Khan).

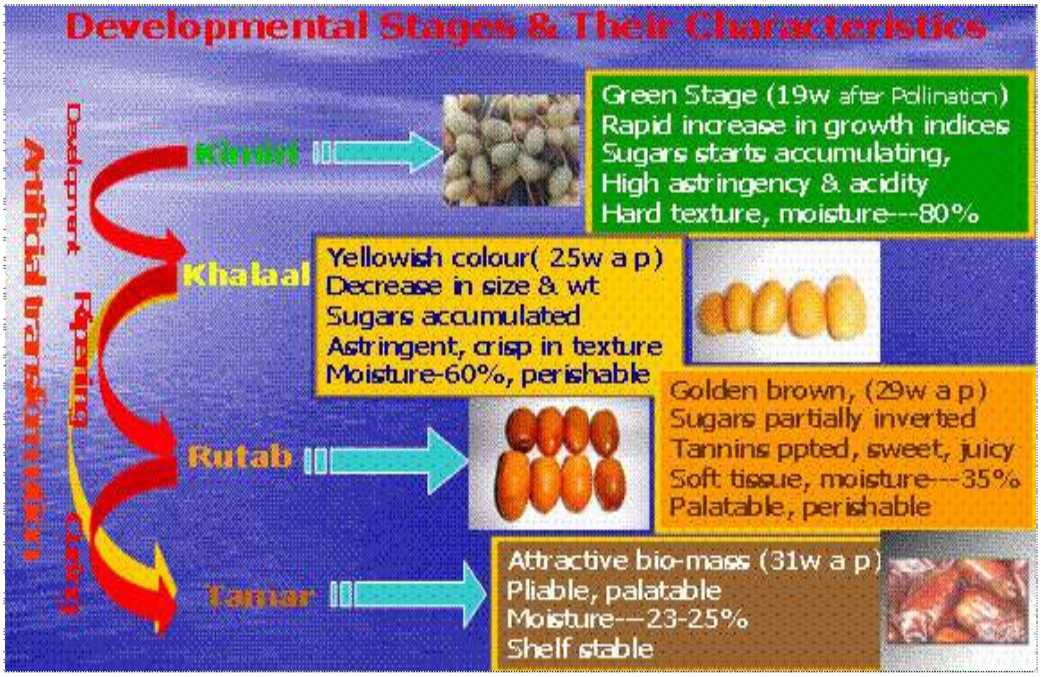

Fig. 2. Adapted technology for stimulated ripening of 'Dhakki' dates showing also the developmental and ripening stages along with fruit characteristics. Note: 'wap' stands for weeks after pollination.

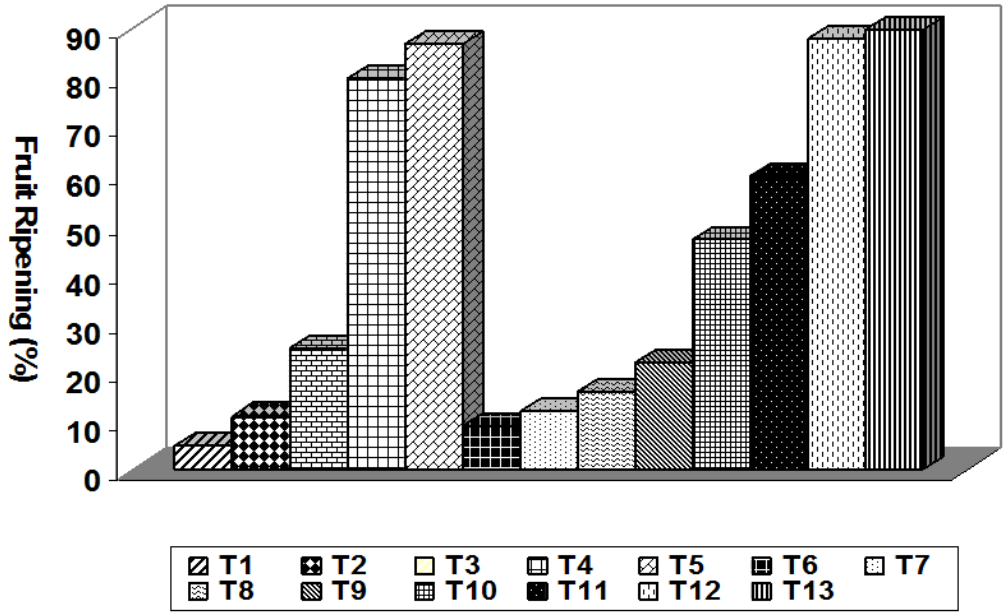

Fig. 3. Effect of artificial ripening on ripening percentage of 'Dhakki' dates.

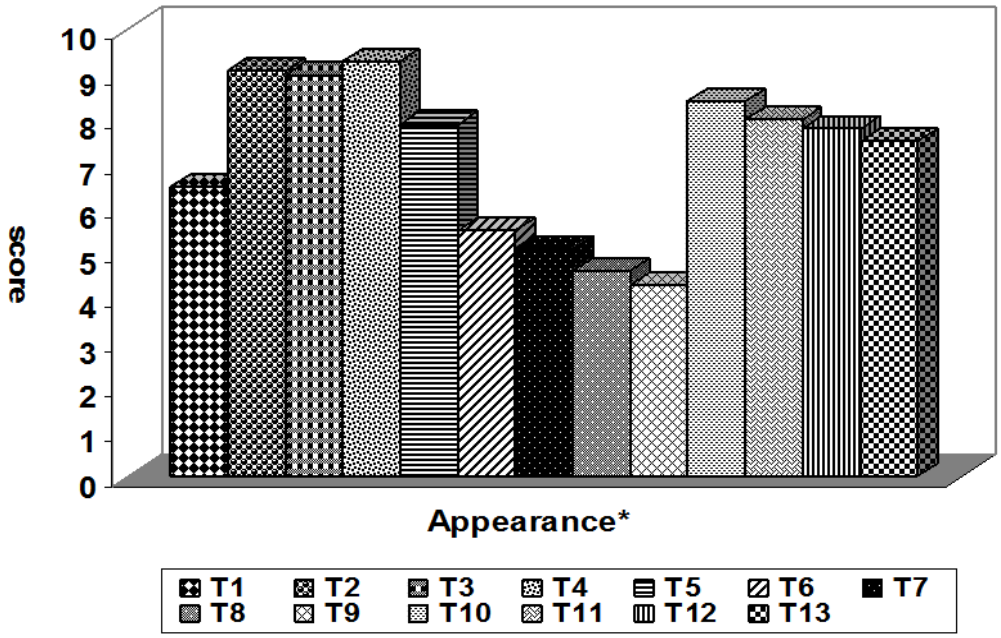

Fig. 4. Organoleptic qualities of 'Dhakki' dates as affected by artificial ripening.

Effect of Using Date Stone Meal Supplementation on Growth Performance, Blood Constituents and Carcass Characteristic of Growing Rabbits

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Keywords: rabbit, date stone meal, growth performance, digestibility trail, carcass traits, blood constituents

Abstract

A total number of 64 flender weaned male rabbits aged 6 weeks with nearly equal live body weight were used in the present search to study the effect of replacing yellow corn by date stone meal in growing flender rabbit diets. Rabbits were randomly divided into four equal groups with four replicates of four rabbits weighing about (645-658 g) as an average.

The first treatment was fed the basal diet as a control while the other three treatment diets were formulated by replacing 10, 20, 30% of yellow corn in the control, the growing rabbits' diet contained 17% CP and 2600 K cal DE/kg diet. All experimental diets were iso-nitrogenous and iso-caloric. The result obtained in live weight and weight gain value significantly varied ($p<0.05$) among different experimental groups of rabbits fed on date stone. Feed conversion ratio (g feed/g gain) revealed a significant difference ($P<0.05$) among the experimental groups. The carcass traits did not differ statistically among the different feeding groups. Digestibility coefficient of DM, OM, CP and CF values significantly varied ($P<0.05$) among the different experimental groups. Dressing percentage and blood constituents were not significantly different.

Generally, from the nutritional and economical point of view date stone could be used successfully and safely up to 30% of rabbits diets without adverse effect on performance, carcass characteristics, meat quality and blood constituents.

INTRODUCTION

The economical world case now is suffering from a strong decrease in elements and income. Arab countries such as Egypt and Palestine are consider as developing countries and they are affected by the economical world case. They suffered a pronounced deficiency in animal feedstuffs. The feed cost of animal nutrition represents more than 70% of the total production cost. It is now urgent to look for alternative feedstuffs to compensate the high cost of the conventional feedstuffs. Egypt and Palestine produce a high amount of palm dates. Date stone meal (DSM) could be used as substitute for yellow corn in rabbit diets. Especially since rabbits are herbivores and consume high fiber diets. They are hind-gut fomenters and are capable of retaining small fiber particles for digestion (Ehrhein et al., 1983). Some experiments were carried out on the use of DSM as an ingredient in rabbits feeding. Most of these experiments showed that DSM can be included in the diet of growing rabbits up to 15% without any diverse effect on productive performance and economical efficiency. This work aimed to study the effect of replacing yellow corn by date stone meal in growing flender rabbit diets.

MATERIALS AND METHODS

This study was carried out at the Al-Ahlaya Society for the Development of Palm and Dates, Der Al-Balah, Palestine, and chemical analysis were carried out at the Animal Production Department (Nutrition), Faculty of Agriculture, Cairo University, Giza, Egypt, during the academic year 2008-2009.

Date stone meal (DSM) was purchased from a Palestinian market, ground to small particle size (8 mm) using a Wiley Mill, then dried in a force draft oven at 65°C for 24 h and then ground in a hammer mill before mixing to the diets.

64 flender weaned male rabbits aged 6 weeks with nearly equal live body (645-658 g) weight were used. Rabbits were randomly divided into four equal groups of 16 rabbits for each in 4 replicates (4 rabbits for each). The first treatment was fed the basal diet as a control while the other three treatment diets were formulated by replacing 10, 20, 30% of yellow corn in the control, the growing rabbits diet contained 17% CP and 2600 K cal DE/kg diet. All experimental diets were iso-nitrogenous and iso-caloric according to the National Research Council (1977) as shown in Table 1. The experimental rabbits were housed in individual batteries provided with feeders and drinkers. For 8 weeks rabbits were weighed at weekly intervals during the experimental period. At the same time, feed consumption and feed conversion were recorded. At the last week of the experiment, digestion trials were carried out by using 3 rabbits from each group to determine the digestible coefficient.

At the end of the experimental period, three representative male rabbits from each group were randomly chosen and fasted for 12 h before slaughter according to Blasco et al. (1993) to determine the carcass traits and blood samples were collected. Serum was separated and used for determination of total protein (Peters, 1968), albumin (Dumas et al., 1971), total lipid (Zollner and Kirsch, 1962), total cholesterol (Wgbenga and Inkpen, 1974), triglyceride (Fossati and Prencipe, 1982), transaminase (AST, aspartate aminotransferase and ALT, alanine aminotransferase) (Reitman and Frankel, 1957) and creatinine (Husdan and Rapopart, 1968) using commercial kits. Proximate analysis was carried out on representative samples using the conventional methods of the Association of Official Analytical Chemists (1990).

All data were subjected to analysis of variance using the general linear models (SAS, GLM), main effect differences obtained upon statistical analysis were compared using Duncan's multiple range test (Hoppe and Dunnett, 1993).

RESULTS AND DISCUSSION

Chemical Composition of DSM Compared to Yellow Corn (DM Basis)

Chemical analysis of DSM in comparison to the yellow corn showed that DSM contained comparable crude protein (6.96 vs. 9.21% and energy content (3468 vs. 3853 K cal/kg), higher CF (12.38 vs. 2.89%), as compared with yellow corn.

Growth Performance

Results in Table 2 show growth performance of flender rabbits as affected by date stone during the experimental period, compared with the control group. Average live body weight and weight gain values were significantly ($p<0.05$) decreased at levels 20 and 30% of DSM while, an insignificant decrease was observed at level 10% DSM. It is interesting to notice that feeding rabbits all the levels of DSM had no adverse effect on weight gain. The previous parameters led to the same previous conclusion by Soliman et al. (2009) that reported that rabbits could tolerate up to 25% DSM in their diets, without adverse effects on their productive performance. These results were in agreement with those reported. On the other hand Aboul-Ela et al. (1999) found that 20% DSM in the rabbit diets decreased both LBW and BWG, significantly when compared to the control.

However, daily feed intake and feed conversion values were insignificantly increased by using all levels of DSM. The results concerning to feed conversion values and the economic efficiency values are shown in Table 2 as well. The results show that 30% DSM resulted in a higher net return value, when compared with other groups.

Nutrients Digestibility

The results in Table 3 show the digestion coefficients of DM, OM, CP, CF and EE. There was significant variation ($P<0.05$) among the different experimental groups.

There were no significant differences in DM, OM and CF digestibility values among the experimental groups. There was a reduction in digestibility values among all groups due to increasing of DSM levels in the diet.

In this regard, Soliman et al. (2009) found that the digestion coefficients of DM, OM, CP, CF, EE and NFE were not affected significantly ($P \leq 0.05$) by using date stone at levels of 15, 20 and 25%. However, Gabrial et al. (1981) reported that the reduction in digestion coefficients due to DSM inclusion could be attributed to the high level of non-starch polysaccharides (NSPs) like cellulose and pentosans (arabinoxylans and glucans), where these components constitute more than 30% of crude fiber (Lennerts, 1988). Also, it was obtained that the decrease in the digestibility of CP values may be due to the low biological values of date stone protein.

Carcass Characteristics

The average pre-slaughter weight and empty carcass traits including dress, head, kidney, liver and heart percentage were not significantly different among tested groups compared with the control group (Table 4). The same results were found with edible parts and non-edible parts. The same results were found by Soliman et al. (2009) who found that carcass traits as affected by different levels of DSM up to 25% recorded no significant difference ($P \leq 0.05$) in carcass characteristics. In this regard, Ibrahim et al. (2004) found that dressing percentages of rabbits fed on diets containing DSM replacing yellow corn up to 100%, were approximately like those of the control groups.

Blood Constituents

The values of plasma total protein, albumin, glucose, cholesterol, triglyceride, urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the treatment groups are shown in Table 5. Results reveled that there was no significant difference in blood constituents among the groups fed different levels of DSM.

CONCLUSION

It could be concluded that flender rabbits can tolerate up to 30% DSM in their diets without any adverse effect on the performance and using different levels of DSM in the rabbit diets improves the economic efficiency.

ACKNOWLEDGEMENTS

Cairo University, Faculty of Agriculture, Egypt, the Faculty of Agriculture, Al-Azhar University, Gaza Palestine and the Al-Ahlaya Society for the Development of Palm and Dates, Der Al-Balah, Palestine are gratefully acknowledged.

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Tables

Table 1. Composition and chemical analysis of the experimental diets.

	Levels of date seed (%)			
Ingredients (%)	Control	T1 (10)	T2 (20)	T3 (30)
Date stone meal	0	10	20	30
Yellow corn	30	20	10	0
Soy bean meal (48%)	25	27	29	29
Wheat bran	34	32	30	30
Palm oil	5	5	5	5
Molasses	3	3	3	3
Sod. chloride	1	1	1	1
Lime stone	1	1	1	1
Vit. and min. premix*	1	1	1	1
Total (KG)	100	100	100	100
Chemical analysis determined**				
CP%	17.10	16.90	16.32	16.13
CF%	13.13	15.06	16.8	17.00
EE%	2.97	3.15	3.18	3.90
Chemical analysis calculated: **				
DE (kcal/kg)	2684	2650	2623	2605

* Commercial vitamin and premix contained (per 3 kg premix): 12,000,000 IU Vit. A; 2000,000 IU Vit. D3; 10,000 mg Vit. E; 1000 mg Vit. K3; 1000 mg Vit. B1; 5000 mg Vit B2; 1500 mg Vit. B6; 10 mg Vit. B12; 10 000 mg pantothenic acid; 1000 mg folic acid; 50 mg Biotin; 30,000 mg Fe; 60,000 mg Mn; 4000 mg Cu; 50,000 mg Zn; 300 mg I; 100 mg Co; 100 mg Se; calcium carbonate to 3.0 kg.
** Calculated according to NRC (1977).

Table 2. Growth performance of flender rabbits as affected by date stone during the experimental periods.

Items	Levels of date stone (%)			
	Control	T1 (10)	T2 (20)	T3 (30)
No. of rabbits	16	16	16	16
Initial body weight (g)	645	656	651	658
Final body wt. (g)	1992a	1974ab	1949c	1937c
Daily gain (g/d)	27.50	26.91	26.50	26.13
Feed intake (g/d)	93.51	94.27	96.82	99.61
Feed conversion	3.40	3.50	3.65	3.81
Feed price/1 kg ($)	0.15	0.13	0.11	0.10
Net return ($)	1.41	1.45	1.50	1.53

Mean values in the same row with different letter(s) are significantly different (P≤0.05).

Table 3. Nutrient digestibility of flender rabbits as affected by date stone during the experimental periods.

Items	Levels of date seed (%)			
	Control	T1 (10)	T2 (20)	T3 (30)
DM	74.10^{a}	70.22^{ab}	67.52^{bc}	59.86^{c}
OM	65.05^{a}	60.64^{ab}	59.19^{bc}	58.98^{c}
CP	77.68^{a}	70.26^{b}	69.32^{bc}	64.56^{c}
CF	34.12	32.34	30.33	30.45
EE	66.68^{c}	76.77^{ab}	75.58^{ab}	70.34^{bc}

Mean values in the same row with different letter(s) are significantly different (P≤0.05).

Table 4. Carcass characteristics for slaughter rabbits as affected by feeding date stone meal diets.

Item	Levels of date stone (%)			
	Control	10	20	30
No. of rabbits	5	5	5	5
Live weight (g)	1992a	1974ab	1949c	1937c
Dressing (%)	56.40	56.60	57.00	57.40
Boneless (%)	43.10	43.70	43.90	44.10
Bone (%)	13.30	12.90	13.80	13.30
Liver (%)	2.68	3.01	3.24	3.60
Kidney (%)	0.86	0.88	0.91	0.93
Heart (%)	0.32	0.33	0.32	0.35
Head (%)	6.30	3.40	6.43	6.45
Edible giblets (%)	3.86	4.22	4.47	4.90
Cecam (%)	6.29	6.32	6.38	6.41

Table 5. Biochemical/parameter of flender rabbits fed diets containing date stone.

Parameter	Control	T1 (10%)	T2 (20%)	T3 (30%)
Total protein	6.87	6.3	6.42	7.17
Albumin	2.88	2.8	2.75	3.3
Glucose	89	91	90	93
Cholesterol	79.2	90.1	93.3	86.5
Triglyceride	120	110	115	105
Urea (mg/dl)	21.5	20.3	20.1	20
Creatinine (mg/dl)	1.2	1.3	1.1	1.0
AST (µ/L)	68	64	70	71
ALT (µ/L)	67	81	37	77
RBC $(10)^6$	5.0	5.7	5.9	5.5
WBC $(10)^3$	6.2	7.3	6.1	5.9
Hb (g/dl)	10.4	11.2	11.1	11.0
Ht	33	35	36	34

Study on the Effect of the Use of Diets Containing Different Levels of Crushed Date Seeds on Growing Assaf Lambs

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Keywords: date seeds, Assaf lambs, growth performance

Abstract

This study was conducted in the farm of the Al-Ahlyia Society for the Development of Palm and Dates (ASDPD) to use the remnants of palm trees (unripe dates, date seeds and palm leaves) in feeding animals (sheep, goats and rabbits) in order to find out the possibility of using concentrated feed as alternative to manufactured fodders and replacing traditional food. We used 20 crossbred Assaf lambs (a breed from 5/8 Owasi×3/8 Freisian) with an average body weight of 27± 2.0 kg which were divided randomly into four equal experimental groups (4 lambs each) to investigate the effect of including date seeds and palm leaves in diets on the growth performance of growing crossbred lambs. The feeding trial lasted for 120 days (1 May-3 September 2009). The animals fed restricted roughage of 600 g/day chopped palm leaves, while concentrated feed mixture (CFM) was offered ad lib (control). Crushed date seeds substituted 50, 75 and 100% of corn and barely in CFM in diets of T1, T2 and T3, respectively. Soybean meal was used to adjust the protein content in treated diets, so the experimental rations were iso-caloric and iso-nitrogenous. The concentrate: roughage ratio was 85:15 approximately. We weighed animals every 2 weeks. Blood samples were taken monthly to assess the effect on animal health. We studied the average growth rate and body changes of the animals, quality and characters of the animal meat and quality and body build of the animal, biochemical and hematological change of the blood, digestibility of the food and the economic effect.

The results showed an increase in the animal weight in T1 and T2 compared with the control during the period of study while there was no difference between the control and T3. The average weight was 283.3-300 g daily. and this increase is a standard by previous studies. There was no difference in the actual weight between the control and T2, T3 but the control with T1. There were no significant differences between the treatments compared with the control in the growth rate only that there was a daily increase in T1 compared to the control.

About the effect of feeding on carcass trials of male sheep lamb, its clearance ratio was almost similar in all groups (50.43, 50.05 and 49.66%) including the control (50%). We observed that the food given was useful and nutritious to the animals. When we studied the muscle mass, bone thickness and fat in all the groups we have observed similarity in all groups in distribution but extra fat in T1 (221.12 g) about 28.86% of fat increment in the control and other groups. From another side there is no difference in the blood picture (Hgb and HCT) in all groups but only little not effective difference, even if there was a better blood picture.

Economically we found less cost in group T3 than in the control group ($ 1.1 in T3 and $ 1.53 in the control/cost of 1 kg of increment in animal weight).

Regarding digestion we found there is an increase in the average digestibility in T1, T2 and T3 compared with the control group and there was improvement in protein and fiber digestion.

Conclusion: we conclude from this study that it is possible to bring a full component of the diet of some commercial palm waste to feed animals (sheep and goats) without any adverse effect on their life and there was a clear reduction in the cost of food to produce kg of live weight of sheep and goats and it can be concluded

that substitution of corn and barley by crushed date seeds can be used to improve the growth performance of Asaf lambs.

INTRODUCTION

Under the circumstances, the economic and political climate and shrinking agricultural land and water scarcity, the repeated sieges in the Gaza Strip since a long time, and the high costs of imported materials, including raw materials, fodder and high costs for transfer to feed factories in Gaza, feed prices are high and in some cases feed is not available as a result of closures and siege, which leads to very high cost of feed for feeding animals such as sheep, goats, rabbits, etc. In the Gaza Strip there is a huge waste of agricultural products available, especially palm waste and remnants of food processing and other, which serve as untraditional alternative fodders, at variable rates, especially in the present time.

Here the focus of the research was on the use of agricultural residues of the palm through the Al-Ahlyia Association for the Development of Palm and Dates and special benefit from the intended dates seeds and leaves and other remnants. Date palm seeds are considered one of the major residues of manufacturing dates for the initial consumption, such as consumer paste dates or fresh dates. Iraq and the Gulf states and Egypt produce date seeds in large quantities. There is a significant expansion in the cultivation of palm trees in Palestine and in the Gaza Strip in particular, where the number of trees is 85000 productive trees and close to 100000 non-productive. The quantity of waste about is 500 tons, including the date palm seeds, in addition to about 800 tons of non-mature-dates.

The dates seeds represents about 25% of the fruit Albanna and Eid (2007). The chemical composition is different of the nuclei by crop type, but this difference is simple, and we can say that the chemical composition in general on the basis of dry matter is: organic matter (90.19-97.8), crude protein (7.8-9.61), crude fat (2.88-4.9), crude fiber (11.25-15.5), and soluble carbohydrates (62.14-73.8), crude ash (2.2-4.01) as previous studies showed (Sawaya et al., 1984; Youssef and Fayed, 2001; Awadalla et al., 2002). From these figures we intended date palm seeds are rich in carbohydrates and to some extent in fat and protein. For this dates seeds are considered as one of the alternatives in animal feed. It has been shown in studies that dates have a positive effect on the weight growth rates of animals and likely feeding has to do with the presence of some growth hormones in the core material, and these help to increase animal growth rates by increasing the level of the amino acids in blood (Alhayshah et al., 2001).

As shown in many studies, the total digested compounds (TDN) in date seeds was high and the increase of the intended dates in the feed caused an increase in the total output of volatile fatty acids in sheep, especially propionic acid. Also, the nutritional value of intended dates is high and placed on the same rank with barley and yellow corn in terms of energy despite the cheap prices and its availability as a waste in the manufacturing of food (Awadalla et al., 2002).

Objective of the Study

To use the remnants of date palm (date palm seeds, leaf, immature date, remnants of others) in feeding sheep, goats and rabbits, and to find out how to use the concentrated feed alternative.

MATERIALS AND RESEARCH METHODS

This study was conducted in two major phases.

Phase I

An analytical study of remnants of palm in the region after a machine had been designed, especially for such waste. 8 samples of this waste were taken, crushed and shredded (dry leaves, green leaves, leaf without pennie, base leaves, fiber, dry branches, seeds, immature dates) in order to determine the proportion of protein and some important nutrients in animal feed, especially for sheep and goats. The samples were sent to the Al-

Azhar University laboratory for analysis of food and feed.

Phase II

Implementation of the first integrated scientific study this study included:

1. The purchase of 12 lambs at the age of 4 months, ranging in weight (27-30 kg).
2. The bush was installed on the target for the experiment which is to bring waste palm dates instead of corn and barley, the level of protein and energy in the diets were approximately equal in proportion in diets replacement rate (0, 50, 75, 100%) on the respectively, of the proportion of barley and corn in each transaction.
3. The work of the chemical analysis of the components that are configured rations were calculated by the ratio of protein and total digestible food ingredients (TDN) in each diet, as well as calculate the proportion of protein digested (CP).

Account the components of the bush on the basis of dry material, as well as calculated by the organic matter (OM), crude fiber (CF) and the ethereal extract (EE) and ash (ASH).

4. The animals are weighed every two weeks to follow up the weight increase and weight gain and increase in weight per day.
5. Blood samples were taken from animals (sheep) every month to study the effect of food on the health status of animals. The number of samples analyzed was about 100. Each sample analysis included the installation of the 15 components of the blood.
6. The estimated average amount of feed eaten per day, per head, based on the decisions (NRC) and the proportion of coarse material from the straw palm leaves. The total edible article of diet was calculated for each application per day.
7. At the end of the experiment was a digestion experiment of animals, selected for three animals from each group. Dung was collected for 3 days in order to study how food within the animal body was digested.
8. After the completion of the experiment the animals (sheep) were slaughtered and the basic components in the animal body were studied, especially the net carcass weight, the weight of edible parts represented in the liver and spleen, kidneys and testes and the weight of non-edible parts represented in the internal organs and fat and skin, leg and head and this in order to know the impact of food on these components and how animals use them.
9. Samples were taken to assess the level and rate of fattening in animals through so-called eye muscle.
10. Samples were taken from the front and back of the parties to estimate the amount of meat, and learned that the benefit of rights and assessment of the bones in the body of the animal.
11. The economic efficiency of the process of animal feed on the remnants of palm was calculated and the economic return to the use of such waste was considered and what is the level of waste palm that can be used in animal nutrition and what are the economical effects.

RESULT AND DISCUSSION

Analysis and Synthesis of Animal Diets

1. Analysis of Remnants of Palm. The results of the analysis which was conducted in the Al-Azhar University Lab. for the Analysis of food and feed in Gaza, are shown in Table 1. The nutritional value, found in the remnants of palm dates, especially in the palm seeds and dry, green leaves is rather high as the percentage of protein in the nuclei of dates is around 7.69, followed by dry and green leaves respectively 4.49 to 4.12 and the percentage of carbohydrates (sugar dissolved) in the seeds of 64.92, followed by immature dates (61) where this ratio is a high source of energy for animals. We conclude from this study that date palm seed and palm leaves can be inserted in the manufacturing of animal feed and there is a need to add materials containing high protein forage such as sesame or grain or soybeans to offset the drop in protein in the nuclei of dates (Albanna

and Eid, 2007).

2. Installation and Analysis of the Components of Diets. Bush has been installed on the target for the experiment which is to bring waste of date palms instead of yellow corn and barley, to the level of protein and energy in the rations approximately equal to the ratio of replacement diets (0, 50, 75, 100%) respectively, of the proportion of barley and corn in each treatment, according to Table 2. From the results of chemical analysis of the components of diets, which showed similar rates of protein in diets that are configured on the basis of dry material as well as components of total digested food (TDN) were respectively (69.75, 69.23, 63.6, 61.35) (Control, T1, T2, T3) also showed the results of chemical analysis for each of the protein digested (CP), as well as organic matter (OM), crude fiber (CF) and the ethereal extract (EE) and ash (ASH), convergence rates for those treatment. It is clear from this analysis that the nutritional value of date seeds within the (T3) containing 57% palm residues is high placed in the same place with barley and corn in terms of energy and this has been the work of bringing the insert waste instead of corn and barley as in Table 3 (Sawaya et al., 1984; Youssef and Fayed, 2001; Awadalla et al., 2002).

Weighted Growth Rates of Animals on the Experience and the Economic Cost

Seen through the Table 4 the weighted rate of increase during the test period was 120 days (34, 36, 35, 34 kg), respectively, of the control group to treatment groups T1, T2 and T3. Where we find that there is an increase of 1-2 kg per treatment (T1, T2) for the comparison group, the increase in T3 is equal to the increase in the total control unit, and this indicates that the sheep responded equally from the remnants of palm seeds as compared with the control and replacement rates used in the experiment had god implications and have not shown any decrease in the rate of increase of the weighted coefficients.

Looking at the daily rate of increase, we find 283.3-300 g per day and these rates are considered standard by previous studies. There are no significant differences in the rate of increase but there is a daily increase in the treatment T1 compared with the control. When calculating the amount of food eaten it was found that there is a decrease in the proportion of food eaten in the treatment compared with the control but the amount of these differences is not significant.

The conclusion of the study is that dates nuclei have a positive effect on the weighted growth rates of animals feeding and is likely to be linked to the presence of some growth hormones in the core material, and these help to increase animal growth rates by increasing the level of the amino acids in the blood. (Kholif et al., 2001).

When calculating the cost per kg of diet in various treatments, compared with the control we found that there are significant differences between the control diet compared with treatments. The cause of the replacement of corn and barley by palm waste is high prices, according to Table 5 where we note a significant decrease in the cost of an increase of live weight and where we find that the control group cost was $ 1.53 while the T1, T2 and T3 were $ 1.42, 1.21 and 1.1, respectively. We can say that the low cost of the increase in weight per kg of live weight is economically viable, especially in light of the availability of such waste in the Gaza Strip (Mohamed and Farghaly, 2002; Awadalla et al., 2002).

Impact on Carcass Characteristics and Eye Muscle

As shown in Table 6 on the impact of treatments on the carcass traits in sheep, its clearance ratio was almost similar in all groups (50.43, 50.05 and 49.66%) including the control (50%). We observed that the food given was useful and nutritious to the animals. When we studied the muscle mass, bone thickness and fat in all the groups we have observed similarity in all groups in distribution but extra fat in T1 (221.12 g) about 28.86% of fat increment in the control and other groups. This gives a clear picture that the food that was being eaten was beneficial showing from well-distributed red muscle and fat on the body, regardless of the type of food eaten.

According to Table 7 in examining the eye muscle and level of bone and fat and muscle therein, the result showed that the distribution of these components in the eye muscle was very close and equal to an increase in the level of fat in T1 compared with the control and T2, T3.

We conclude from this study that there were no adverse effects on carcass characteristics when using palm waste as alternative to non-traditional barley and corn samples in treatments as we can deduce that there is a clear impact of the structures of feed on the fat percentage and this is likely to be linked to the presence of some growth hormones in article cores, and these help to increase animal growth rates by increasing the level of the amino acids in the blood (Abd El-Raman et al., 2003).

The Blood Picture in Sheep and Chemical Analysis

Table 8 shows the blood picture of sheep to study the impact of the use of such waste on the number of red blood cells, hemoglobin and alheimotocrit and the number of white blood cells. We found that there are simple but significant differences between the various transactions, especially in the average number of red blood cells and the percentage of alheimotocrit and hemoglobin were higher in terms of transactions reported in the control group. Thus there are no adverse effects on the use of such waste, but improvement in the rates of blood picture structures. And according to Table 9, which shows the work of chemical analysis to study the blood of sheep, if there are bad influences of the use of these wastes listed in nutrition, we have been examining the level of protein and sugar and liver functions and functions of the college and triglycerides and cholesterol and the results were all similar in comparison with averages compared with the control group. There were no significant differences and there were no adverse effects on animals through the identification of the chemical composition of blood.

We can conclude from the above mentioned that the alternative diets have no adverse effect on experimental animals, but improved rates of blood, or combinations of images of the chemical composition (Kholif et al., 2001).

Transactions of the Digestion of Nutrients

The results were as shown in Table 10, where most transactions increased digestion of nutrients as well as the nutritional value of a digestible nutrients (TDN) 64.3, 66.7, 57.4 and 53.17 (Control, T1, T2 and T3), respectively, and digested protein (DCP), respectively, 9.3, 8.85, 8.83 and 7.2 (Control, T1, T2 and T3) significantly in each of the bush first and second as compared to the comparison factor has also increased digestion of crude protein, crude fiber. Drawn from the foregoing it can reduce the cost of food for sheep when you use certain processing waste in non-traditional diets. The efficiency of food manufacturing is high when feeding on bush (3), followed by bush (1) and (2).

The cost of food to produce 1 kg growth was as high as possible with the first diet and then the second and third) (Mohamed and Farghaly, 2002; Abou El-Naser and El-kerdawy, 2003).

CONCLUSION

We conclude from this study that it is possible to bring a full component of the diet of some commercial palm waste to feed animals (sheep and goats) without any adverse effect on her life and there was a clear reduction in the cost of food to produce kg of live weight of sheep and goats and it can be concluded that substitution of corn and barely by crushed date seeds can be used to improve the growth performance of Assaf lambs.

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Tables

Table 1. Nutritional value in the remnants of palm.

Item	cp	fat	fiber	ash	Sug	hum
Dry leaves	4.49	2.12	29.60	4.39	21.78	32.01
Green leaves	4.12	3.41	26.40	4.11	20.91	38.89
Dry leaves without pennie	0.11	1.12	17.72	2.40	19.30	55.36
Base leaves	1.91	4.13	20.21	5.20	19.60	49.81
Fiber	0.87	1.71	31.20	4.16	33.30	25.16
Branch	0.91	3.15	17.60	3.20	49.36	25.92
Seeds	7.69	2.96	13.94	8.72	64.92	10.42
Immature dates	2.64	1.30	11.40	1.49	61.00	12.62

Table 2. Concentrate mixture formulation.

Concentrate mixture formulation	Treatment			
	Control	T1	T 2	T 3
Yellow corn	20	10	5	0
Barley	20	10	5	0
Soybean	7	10	10	10
Date seeds	0	20	20	20
Date by-product	20	17	27	37
Wheat bran	30	30	30	30
Limes ton	1	1	1	1
Sodium chlorides	1	1	1	1
Vitamin	1	1	1	1
Total	100	100	100	100

Table 3. Chemical composition of the experimental rations (% Dm basis).

Item	DM	OM	CP	CF	EE	NFE	TDN
Control	92.68	86.19	15.0	6.07	3.17	64.27	69.75
T1	93.71	87.13	15.0	7.81	4.38	63.28	69.23
T2	93.02	86.18	15.0	8.78	4.10	63.38	63.60
T3	93.28	84.84	15.0	9.75	5.03	59.47	61.35

Table 4. Average daily feed intake (kg/h/day) of the tested rations during the feeding trial in sheep.

Item	Control	T1	T2	T3
Concentrate feed mixture (CFM)	2.00	2.00	1.88	1.87
Palm hay	0.20	0.20	0.20	0.20
Total (kg/h/d)	2.20	2.20	2.08	2.07

Table 5. Performance of ram lambs fattened on palm by-product.

Items	Control diet	T1	T2	T3
No. of animals	3	3	3	3
Duration of trail (days)	120	120	120	120
Av. initial BW (kg)	33	32	33	34
Av. final BW (kg)	67	68	68	67
Av. total gain (kg)	34	36	35	34
Av. daily gain (kg)	283.3	300	291.6	283.3
Feed conversion (g feed/g gain)	7.65	7.08	7.14	7.29
Feed consumed (kg) to produce total gain	260	255	250	248
Price of kg diet ($)	0.25	0.2	0.17	0.15
Feed cost for kg gain ($)	1.53	1.42	1.21	1.1

Table 6. Effect of feeding the experiment diet on carcass trial of male sheep lambs.

Item	Control	T1	T2	T3
Final body weight (kg)	65.33	65.70	65.33	66.33
Hot carcass (kg)	32.66	33.12	32.70	32.94
Dressing (%)	50.00	50.43	50.05	49.66
Organ % of FBW				
Head	5.40	4.98	4.41	4.70
Pelt	10.10	10.62	10.70	11.25
Feet	2.10	2.29	2.15	2.20
Offals % of FBW				
Heart	0.48	0.41	0.40	0.40
Liver	1.48	1.48	1.40	1.50
Kidney	0.30	0.31	0.36	0.25
Lungs	1.72	1.75	2.17	1.33
Spleen	0.146	0.147	0.26	0.19
Testis	1.16	1.50	1.42	1.00
Fat % of FBW				
Abdominal	3.58	3.70	3.70	3.39
Tail	2.44	1.60	1.43	2.99
Kidney	2.32	1.26	1.10	1.87
Whole sale cuts % of FBW				
Neck	5.54	5.51	5.52	5.61
Shoulder	9.97	9.90	9.92	10.00
Loin	6.92	7.00	7.01	7.10
Flank	3.36	3.50	3.51	3.60
Hind legs	15.90	16.10	16.00	16.2

Table 7. Ribs 9, 10, 11 wt. (g) and percentage of meat, bone and fat (% hot carcass) and eye muscle area (cm^2 wt.) as affected by type of diet in sheep.

	Control	T1	T2	T3
Number of animals	3	3	3	3
Hot carcass	32.66	33.12	32.7	31.94
Ribs 9, 10, 11 wt. (g)	726.21	766.13	728.15	715.16
Ribs (%)	2.2	2.3	2.22	2.23
Meat wt. (g)	408.85	416.60	383.06	373.74
Bone wt. (g)	148.54	128.53	140.72	120.10
Bone (%)	20.49	16.77	19.32	16.8
Fat wt. (g)	137.9	221.12	143.15	172.21
Fat (%)	18.99	28.86	19.66	24.08
Eye muscle area (cm^2)	37.85	31.52	31.81	27.22

Table 8. Hematology blood picture in sheep.

	Control	T1	T2	T3
RBC $(10)^6$	5	5.6	4.9	5.2
HB (g/dl)	12.5	13.5	12.4	13
Hct	32	34	33	34
WBC $(10)^6$	5.2	4.5	4.4	6

Table 9. Effect of feeding the experimental diet on some blood parameters of tested animals (sheep).

Parameter	Sheep			
	Control	T1	T2	T3
Total protein	7.24	7.32	7.29	7.12
Albumin (g/100 ml)	3.68	3.69	3.74	3.58
Globulin (g/100 ml)	3.56	3.63	3.55	3.54
A/G ratio	1.03	1.02	1.05	1.01
Glucose (g/100 ml)	61.38	63.3	64.5	64.83
Gpt (iu/L)	8.72	8.38	8.63	8.78
Got (iu/L)	61.32	62.72	62.83	64.63
Cholesterol	148.7	150.2	149.6	148.8
Triglycerides	102.3	115.6	107.3	109.2
Greatinine (g/100 ml)	1.15	1.01	1.06	1.08

Table 10. Apparent nutrients digestibility and nutritive value of the experimental dials feed to growing lamb sheep.

Item	Sheep			
	Control	T1	T2	T3
Dry matter (DM)	53.78	54.79	57.86	58.83
Organic matter (OM)	68.30	70.10	69.70	70.20
Crude protein (CP)	56.18	58.25	60.37	61.31
Crude fiber (CF)	54.01	50.65	50.43	49.65
Ether extract (EE)	76.66	77.25	78.11	80.69
Nitrogen free extract (NFE)	56.57	60.11	69.32	65.20
Nutritive value % TDN	53.17	57.40	64.30	66.78
DCP	7.20	8.83	8.85	9.30

Figures

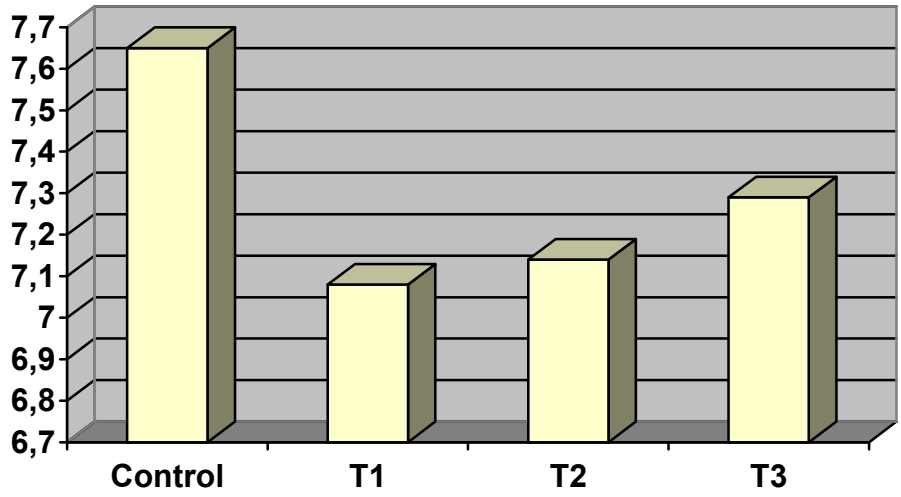

Fig. 1. Food conversion rate during the test period for various treatments.

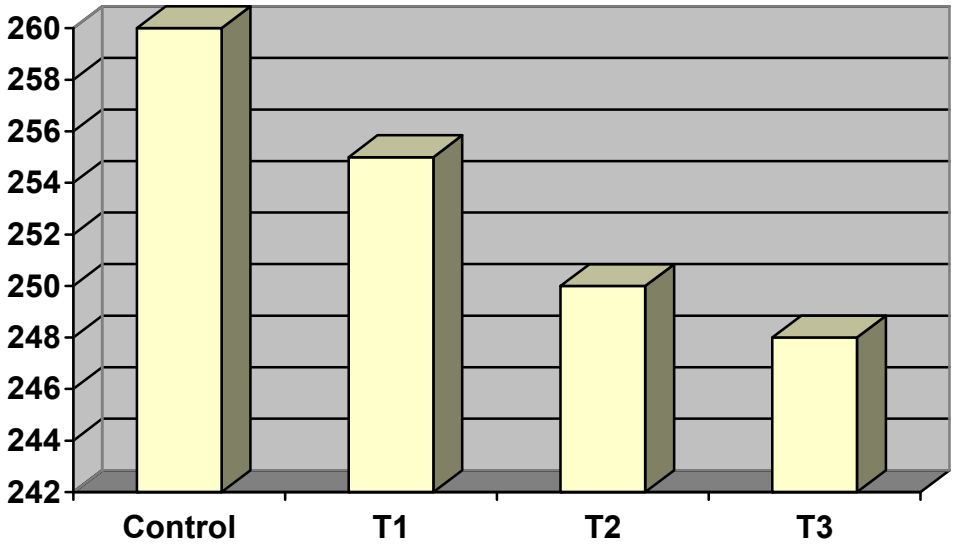

Fig. 2. The average amount of food eaten during the test period for various transactions in sheep.

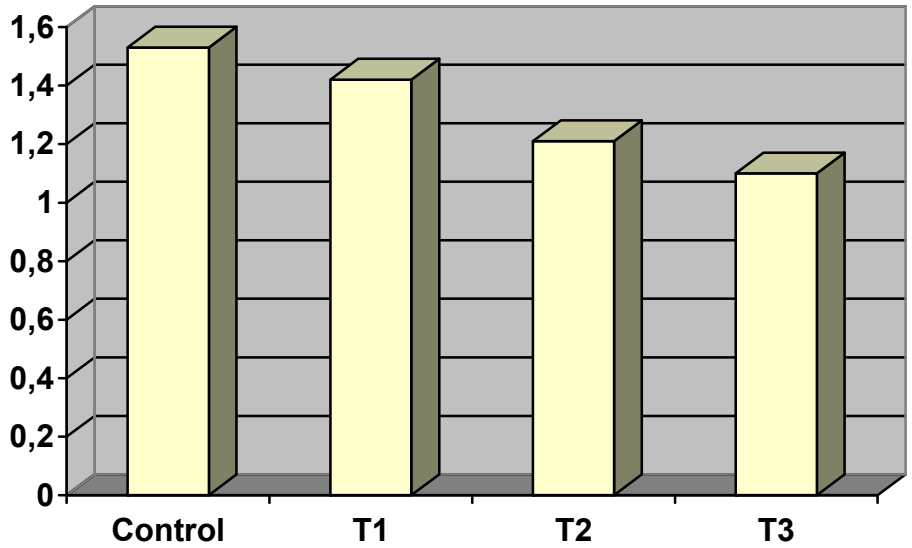

Fig. 3. The economic efficiency during the test period for various transactions in sheep.

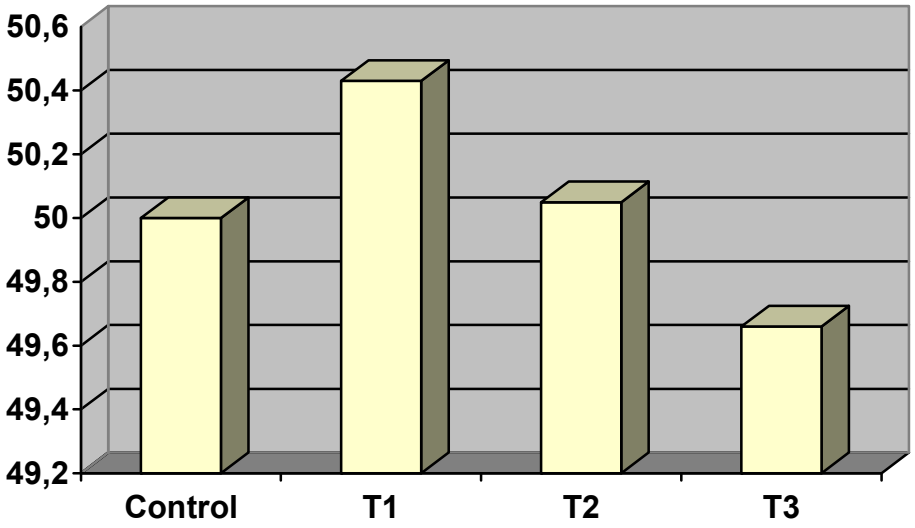

Fig. 4. The ratio of the percentages of dressing in the carcass during the trial period for transactions in the various sheep.

The Marketing and Consuming of Date Palm (*Phoenix dactylifera*) in Turkey

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Keywords: date palm, *Phoenix dactylifera*, in export, marketing, consumption, Turkey

Abstract

The palm (*Phoenix dactylifera*) is imported from the countries Saudi Arabia, Iran, Tunisia, Israel (Jerusalem) and South Africa. The date palm market is mainly composed of the products from Saudi Arabia. The date palm from Medina is the first in respect of the quality. The second and the third are the date palm from Jerusalem and Tunisia respectively. The date palm from Iran is low in quality and it is mainly consumed with low prices in Ramadan month. The date palm from Iran is first washed and then serviced in big shopping centers during the Ramadan. It is supposed that 300 tons are sold during the period. Since the date palm imported from Iran is washed and serviced in big shopping, it becomes mouldy in a short time. There is also another date palm market which is composed of shocked date palm packets. The date palm is composing of 40 types and dates are mainly consumed in big cities such as Istanbul, Ankara, Konya, Kayseri and Bursa. The high season of consuming the date palm in Turkey is the pilgrimage period. The date palm input from Saudi Arabia during the period is legally nearly 800 tons. Additionally, the pilgrims are also thought to bring 6000 tons palm from Saudi Arabia while coming back to Turkey.

INTRODUCTION

Date is a fruit grown in warm climates. Water and high temperature are necessary for growing of dates which exist for 8 thousand years. Even though the dates which are planted at watery areas grow tall, they do not fructify if there is not enough of temperature. In Turkey date is used in parks, gardens and road afforesting for decorative purpose in the Black Sea, Marmara, Aegean and Mediterranean Regions which are marine climate dominated and Southeastern Anatolia Region which is considerably hot during the summer months. However, since this plant finds enough water but does not reach enough temperature totals, it blooms but its fruit cannot grow mature enough in Turkey. Accordingly, Turkey has to import dates from grower countries. Turkey imports dates from Saudi Arabia, Tunisia, Israel (Jerusalem), Algeria, South Africa and Iran. Among these countries, the dates imported from Saudi Arabia compose predominantly the date market area.

TURKEY MARKET AND SELLER COUNTRIES

The Medina dates, the best in terms of quality, compose the majority of dates which enter into Turkey. Jerusalem and Tunusia dates follow this type. In Ramadan month, a certain amount of dates are imported from Iran.

In Turkey most consumption is during hadj and umrah period, Turkish merchants import first quality Medina dates, which is directed to this goal. It is known that 800 tons Medina dates are imported into Turkey via legal ways. Every year 200 thousand people for hadj worship and 50 thousand people for umrah go to Saudi Arabia from Turkey. Each single pilgrim brings at least 24 kg dates into Turkey on the way back. Quite often

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this amount reaches 48 and 72 kg. If it is considered that each person brings 24 kg dates, it will be understood that at least 6000 tons dates come into Turkey with pilgrims via unrecorded ways. The pilgrims going to blessed lands bring dates and zam-zam water for a treat on the way back. Especially buying and carrying dates are comparatively troublesome. Turkey Religious Affairs Administration and hadj organization companies, considering the types which pilgrims prefer, see importing the dates which pilgrims bring with them on the way back as an important issue. Besides Saudi Arabia, date importation is carried out into Turkey from Jerusalem, Tunisia, South Africa and Iran. That particularly during the Ramadan month from Iran around 300 tons date enter to Turkey and these dates are lower quality compared to other date types that are seen. These kinds of dates, which are low quality namely 'Rabbi' in Iran and are assessed as animal feed, are washed and put on the market in Turkey during the Ramadan month. However, they become mouldy depending on dampness.

DATE TYPES AND PRICES IN TURKEY

It has been ascertained that Turkey, where there is also shocked fresh date market, has 40 types of dates, 7 types of wet date, 23 types of processed date products and the most important consumption centers are Istanbul, Ankara, Konya, Kayseri and Bursa. Dried dates can be saved at 4-5°C approximately 10 years. Shocked fresh dates have to be saved at -22°C. It has been estimated that there is 40 tons of shocked fresh dates consumption per year.

Dried Dates

1. 'Hudri'. It is a saporific flavoured, nourishing, hematinicly consumed type of date which is suggested especially to woman and children. 1 kg 'Hudri' is 15.90 TL ($ 1=1.5 TL).

2. 'Mesruk'. It is a delicious and nourishing date type grown especially around Medina, known as Medina date by Turkish people, preferred for hadj and umrah visits as a treat. It is also known as the mostcrusty 'Mebrum' date type and differs from the other types via this way.1 kg 'Mesruk' is 10.40 TL.

3. 'Mebrum Jumbo'. It is a delicious and nourishing date type grown especially around Medina, known as Medina date by Turkish people, preferred the most for hadj and umrah visits as a treat. It is also known as the crustiest 'Mebrum' date type. It differs from the other 'Mebrum' types via its enormity and thin cober. 1 kg 'Mesruk' is 34.56 TL.

4. 'Mebrum 1'. It differs from 'Mebrum Jumbo' by its smaller size. 1 kg is 28.89 TL.

5. 'Mebrum 2'. Its size is much smaller than 'Mebrum Jumbo' and 'Mebrum 1'. 1 kg is 20.80 TL.

6. 'Mebrum 3'. 'Mebrum 3' differs from 'Mebrum Jumbo', 'Mebrum 1' and 'Mebrum 2' with is smaller size. 1 kg is 16.27 TL.

7. 'Şelebi'. It is a medium size, delicious and nourishing date type grown around Medina. 1 kg is 17.28 TL.

8. Big 'Avce' Date. It is grown around Medina and the most special date in terms of the fact that the prophet Muhammed (Peace be upon Him) planted it with His blessed hands, there are 99 names of Allah (c.c.) on it and it was subjected the miracle.1 kg is 108 TL.

9. 'Avce 2'. It is the second quality of 'Avce' date. 1 kg is 69.12 TL.

10. 'Hufri' (Baklava Date). 'Hufri' is a very sweety, big and meaty date type and known for especially its hematinic feature. 1 kg is 27.65 TL.

11. 'Amber'. It is a good-sized, meaty date type having a delicious taste. It is suggested to the ones on a diet in terms of its filling and nourishing feature. 1 kg is 46.22 TL.

12. 'Medjoul'. It is a flavorous, nourishing, coarse-grained date type whose cover colour changes from light to dark brown, and dry weight is between 15-23 g. It is grown especially around Jerusalem and Hejira region of Medina. It is suggested particularly to the children and the old who cannot eat dried and hard date. It is indicated as one of the hematinic date types. 1 kg is 46.44 TL.

13. 'Hilliye'. It is a small-sized, flavoured and nourishing date type named as also the solid state of blood. 1 kg is 23.11 TL.
14. 'Sugi'. It is a delicious and nourishing date type which is preferred by diabetic patients because of the fact that its sugar proportion is low and beneficial in terms of health. 1 kg is 28.67 TL.
15. 'Safavi'. Particularly grown around Medina, preferred because of the fact that it is beneficial for liver and kidney illnesses. 'Safavi' is a delicious date type whose sugar proportion is low. 1 kg is 23.11 TL.

Fresh Dates
1. 'Berni'. Also called as green almond, the most recommended one from the stand point of nutritiousness, taste and health. It is a yellowish date type which is somewhat acrid. It is has to be preserved in deepfreeze or freezer. 1 kg is 13.82 TL.
2. 'Sukkeri'. It is a date type whose name means sugar. Its sugar proportion is much higher compared to the others. It is particularly delicious and curative for the ones who cannot eat dry and hard dates. It has to be preserved in freezer or deep freezer. 750 g of it is 23.11 TL.
3. 'Bied'. 'Bied' date, a delicious and meaty date type, is completely a source of energy and health. It has to be preserved in deep freezer or freezer. 1 kg is 23.11 TL.
4. 'Helva'. Resembling plum, very nourishing, delicious, and somewhat acrid and sweet scended. 'Helva' is a date type that Arabs offer to their guests. It has to be preserved in deep freezer or freezer. 1 kg is 23.11 TL.
5. 'Rabia'. Delicious, nourishing, and very beneficial in terms of health. 'Rabia' is a wet date type whose colour is brownish yellow. It has to be preserved in deep freezer or freezer. 800 g of it is 23.11 TL.
6. 'Rotana'. A delicious, nutrimental and healthy date. 'Rotana' is a wet date type which is suitable for eveyone's taste with its brownish orange colour, coarse and soft structure. It has to be preserved in deep freezer or freezer. 1450 g of it is 37.15 TL.
7. 'Maktuva'. A delicious, nutrimental and healthy date. 'Maktuva' is a wet date type which everyone can eat with admiration. It has to be preserved in deep freezer or freezer. 1 kg is 23.11 TL.

SALES CONDITIONS IN A YEAR

Officially, the consumption of dates in a year; 70% during Ramadan month, 20% during hadj period, 10% during other months. Total custom free dates' 80-85% consumption is during hadj period and the rest of it is during umrah and other periods. Only Turkish hadjis buy 'Mebram' dates from Saudi Arabia. It costs 28-30 riyal in the garden. 'Mebrum' date was 8 riyal in 2002, it increased four times within 7-8 years because of the great interest of hadjis especially in this kind of date. It can be said that within a year, prices of date are stable.

USAGE AREAS AND ASSESSMENT FORMS OF DATE IN TURKEY

Today, there are 40 kinds of dried, 7 kinds of fresh dates in Turkish markets. In Turkey, dates are processed in 23 kinds of by-products. Among the by-products in which especially small grained dates are used, molasses, sausage, cezerye, vinegar, jam are in the first place and there are a lot of areas in which they are processed such as nutty, with hazelnut, with pistachio, chocolate coated, with almond. Date is used in the cake industry as well as it is significantly processed in the dairy, ice-cream and candy industry.

DATE PRODUCTS AND SALE PRICES IN TURKEY

As a Milky Product

Milky date (23.11 TL/500 g), milk chocolate (28.89 TL/1 kg), milk chocolate with peanuts (34.56 TL/1 kg), milk chocolate with walnut (34.56 TL/1 kg), milk chocolate

with pistachio (34.56 TL/1 kg), milk Chocolate with almond (34.56 TL/1 kg), milk chocolate with hazelnut (34.56 TL/1 kg), milk chocolate mixed date (34.56 TL/1 kg).

As a Chocolate Product

White chocolate with almond (23.11 TL/500 g), bitter chocolate with date (28.89 TL/1 kg), white chocolate with date (28.89 TL/1 kg), mixed chocolate date (28.89 TL/1 kg), bitter chocolate date with peanut (34.56 TL/1 kg), bitter chocolate date with walnut (34.56 TL/1 kg), bitter chocolate date with pistachio (34.56 TL/1 kg), bitter chocolate date with almond (34.56 TL/1 kg), bitter chocolate date with hazelnut (34.56 TL/1 kg), bitter chocolate mixed date (34.56 TL/1 kg), chocolate mixed date with fruits (34.56 TL/1 kg).

As a Dried Fruit Product

Date with walnut (34.56 TL/1 kg), 'Sugi' with almond (17.28 TL/500 g), 'Sufri' with almond (17.28 TL/500 g), 'Hudri' with almond (17.28 TL/500 g), 'Selebi' with almond (17.28 TL/500 g).

Other Products of Date

1. Date Coffee. Date coffee without caffeine is prepared after cleaning the date cores completely. Then it is baked and ground like normal coffee cores. Its smell and colour is like turkish coffee. (2.31/100 g).
2. Date Cezerye with Hazelnut. It is a new and delicious taste that is prepared with adding hazelnut which is healthy and nourishing that is produced completely from date naturally without adding any foreign substance and sugar (17.28 TL/1 kg).
3. Date Cezerye with Pistachio. It is new and delicious taste that is completely prepared with adding date and pistachio (17.28 TL/1 kg).
4. Date Cezerye with Walnut. It is new and delicious taste that is prepared with only adding date walnut (17.28 TL/1 kg).
5. Date Dipping with Walnut. By adding date into walnut "date dipping with walnut" can be prepared. It is covered with Turkish Delight on the outside of it and there is date paste and a harmonious taste of walnut in the inside of it. It is source of energy because of the walnut, Turkish Delight and date (17.28/1 kg).
6. Date Atomu with Hazelnut. Completely pure cezerye that is acquired from date are rolled into small pieces and it is decorated with delicious hazelnut grains (23.11 TL/1 kg).
7. Date Atomu with Walnut. Completely pure cezerye that is acquired from date are rolled into small pieces and it is decorated with delicious walnut grains (23.11 TL/1 kg).
8. Date Atomu with Pistachio. Completely pure cezerye that is derived from date are rolled into small pieces and it is decorated with delicious pistachio grains (23.11 TL/1 kg).
9. Stuffed Date. It is a delicious and strong product that is prepared with the mixture of curative and delicious 'Sukkeri' date, almond and pistachio paste. It is decorated with puffed rice and consumed with coffee and tea (28.89 TL/1 kg).
10. Date Paste. This delicious product is made of well cleaned fresh date with special spices by adding flour and butter in it. It is very nourishing and it is recommended for especially women who gave birth to increase milk secretion and replace the liquid that is lost during birth (17.28 TL/1 kg).
11. Date Powder. Special date that is used in drug production and its core are dried in the shade. Subsequently, both of them are pestled and pulverized. It is produced as pure, completely natural (11.56 TL/300 g).
12. Date Sugar. It is made of date and sugar (8.10 TL/1 kg).
13. Date Covered with Carob. It is prepared with the combination of carob powder and 'Mebrum' date (11.56/500 g).
14. Date Sausage. It is made of date and walnut (1.73 TL/ 1 packet).
15. Date Paste. Medina dates are ground properly and walnut grains are added in it. It is prepared with sprinkling pistachio outside of it and it does not include sugar or any foreign substances (28.89 TL/1 kg).

16. Konya Wrapping. It is prepared as an alternative to Konya wrapping (6.91 TL/ 400 g).
17. Date Jam. It is prepared with coreless dates in a hygienic environment. 65% of it is fruit (11.56 TL/ 450 g).
18. Date Molasses. It has high nutritional values. Molasses produced from date is lighter and tastier. It is a sweet and intensive syrup that is produced by grinding and boiling fruits. It tastes like chocolate when it is eaten with sesame seed paste (8.10 TL/380 g).
19. Date Cake. It is ready made with date (11.56 TL/1 packet).
20. Date Vinegar. It is made of date fruits (11.56 TL/500 ml).

PALMS USED AS ORNAMENTAL PLANT IN THE PARKS

Palms are commonly used in the coastal cities of north, west and south regions and it is used as a landscape plant in the parks and gardens of southeastern Anatolia intensely. It blooms and produces small fruits in Mediterranean and Southeastern Anatolia. However, it cannot be utilized because of its immature fruits.

RESULT

Turkey is an important country in terms of date market. Some manufacturers in Turkey offer new products of dates to the markets. During hadj and umrah period important amount of products come to Turkey. Officially 800 ton dates importation comes from Saudi Arabia, 300 ton dates importation from Iran were ascertained. It was concluded that instead of bringing dates with them, it will be more suitable that hadj organization companies import dates via customs entry and deliver to the hadjis. In the circumstances, hadjis will not have to waste time to supply and carry dates. It is recommended for The Presidency of Religious Affairs of the Republic of Turkey and hadj organization companies to evaluate this condition.

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Study of the Effects of Storage Methods and Amount of Pollen on 'Zahidi' Date Palms Fruit Setting

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Keywords: pollen viability, 'Zahidi', pollen, pollination, fruit set, fruit drop

Abstract

Bushehr province with 34000 ha of date plantation owns the third area rate of dates production in Iran. 'Zahidi' as a dry cultivar with high handling and storage capacity is the most important date production in this province after 'Kabkab' cultivar. Unfortunately, fruit drop in this cultivar is one of the main problems. The amount of pollen usage and pollen viability are effective factors on pollination. An experiment was done to study the effects of storage methods (fresh pollen, stored pollen at room temperature and stored pollen in refrigerator) and amount of applied pollen (0, 0.5, 1 and 2 cm^3) on fruit set and fruit drop of 'Zahidi' with twelve treatments and four replications in Factorial Randomized Complete Blocks Design. Results showed that the main effect of pollen storage methods was significant on fruit drop, fruit weight, seed weight, length and diameter, bunch weight, TSS, fruit set, ripening time and pH, but showed no effect on length and diameter of fruits. Amount of pollen had no significant effects on any of the mentioned properties. Interaction between storage methods and amount of pollen had significant effect only on fruit drop and pH. Also, it was shown that pollen viability was significantly affected by pollen storage methods at 1% level.

INTRODUCTION

Date palms are dioecious, that is, the male flowers and female flowers are born on separate palms. To ensure satisfactory fruit set and development, female flowers have to be artificially pollinated. The pistillate flower consists of three carpels, only one of which develops when the flower is fertilized. When pollination is prevented or fertilization does not occur, all or one of the three carpels will develop into parthenocarpic fruits (Reuveni, 1986). However, exact investigation on demand of pollen amount in different cultivars is not carried out, but it is believed that farmers overuse pollen. Because of the high price and probably bad effect of high pollen on drops of flowers and fruits, it is sensed that a suitable amount of pollen should be identified (Eata, 1986). Study on storing of pollen related to 1930 when Albert showed that pollen stored in 3.2°C temperature have higher germination compared with room-temperature stored pollen (Albert, 1930). Zargari (2002) reported that fruit set percent in pollinated bunches with 20% pollen and 80% bran or 100% pollen were 52.8 and 43.9% respectively (Zargari, 1992).

MATERIAL AND METHODS

This study was done in Bushehr date palms and tropical fruits research center with sandy soils. This experiment was done to study the effects of storage methods (fresh pollen, stored pollen at room temperature and stored pollen in refrigerator) and amount of applied pollen (0, 0.5, 1 and 2 cm^3) on fruit set and fruit drop of 'Zahidi' with twelve treatments and four replications in Factorial Randomized Complete Blocks Design. Pollen was collected from the 'Jarvis' cultivar.

Cultural media (Brewbaker and Kwak) was used for determination of the viability of pollen in Completely Randomized Design with 4 replications. In order to prevent the entrance of unwanted pollens to spathes, they were covered by cotton bags before their cracking. One or two days after the spathes opened naturally, the treatments were done.

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One week after pollination the bags were opened and dropped flowers were counted and the bags were closed for another week. Then, counting was done again and bunches were uncovered. The number of strands in each bunch and the number of fruits on 10 strands were counted randomized. This work was repeated at the end of every month. The data were analyzed with MSTATC and the means were compared with Duncan's multiple range test (DMRT).

RESULTS AND CONCLUSION

The results showed that pollen viability is affected by storage method of pollen at 1% level. Storing pollen for one year decreased viability, so that the viability which was 76.1% in fresh pollen collapsed to 53% in refrigerator stored ones. The viability of room-temperature stored pollen was less than 5% in the following year. Fruit drops were increased when storage pollen from the previous year was used for pollination in comparison with fresh pollen. The minimum and maximum fruit drop was observed in fresh pollen and room-temperature stored method with 41.3 and 80.9% respectively. The interaction between storage method and amount of pollen proved that the more pollen used, the more drop happened for fresh and room-temperature stored pollen. But, increasing the usage of refrigerated pollen decreased fruit drop.

The maximum fruit and flower drop occurred two weeks after pollination (in March), but then it decreased until August. Preharvesting drop happened in fresh pollen treatment in the first half of August (Fig. 2).

The results also showed that a definite amount of pollen should be used for satisfactory pollination and fruit set which is related to pollen viability. Less fresh pollen with high viability is needed than stored pollen with lower viability.

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Tables

Table 1. Content of culture media (Brewbaker and Kwak).

Chemical matter	Formula	Amount
Boric acid	H_3BO_3	0.5 g
Potassium nitrate	KNO_3	0.1 g
Sucrose	$C_{12}H_{22}OH$	15% W/V
Magnesium sulphate	$MgSO_4$	0.2 g
Calcium nitrate	$Ca(NO_3)_4H_2O$	0.2 g
Diluted water	H_2O	1 L

Table 2. Interaction of storage method and amount of pollen on fruit drop (%).

Storage methods	Amount of used pollen (cm^3)			Mean
	0.5	1	2	
Fresh pollen	39.7a*	40.9a	43.5a	41.3A
Pollen stored at room temperature	74.4de	82.6ef	85.5f	80.9C
Pollen stored in refrigerator	70.6cd	64.4bc	58.9b	64.6B
Mean	61.6A	62.6A	62.6A	

* Means in each column or row with the same capital or small letter are not significantly different at 5% level.

Figures

Fig. 1. Spathes covered by cotton bag.

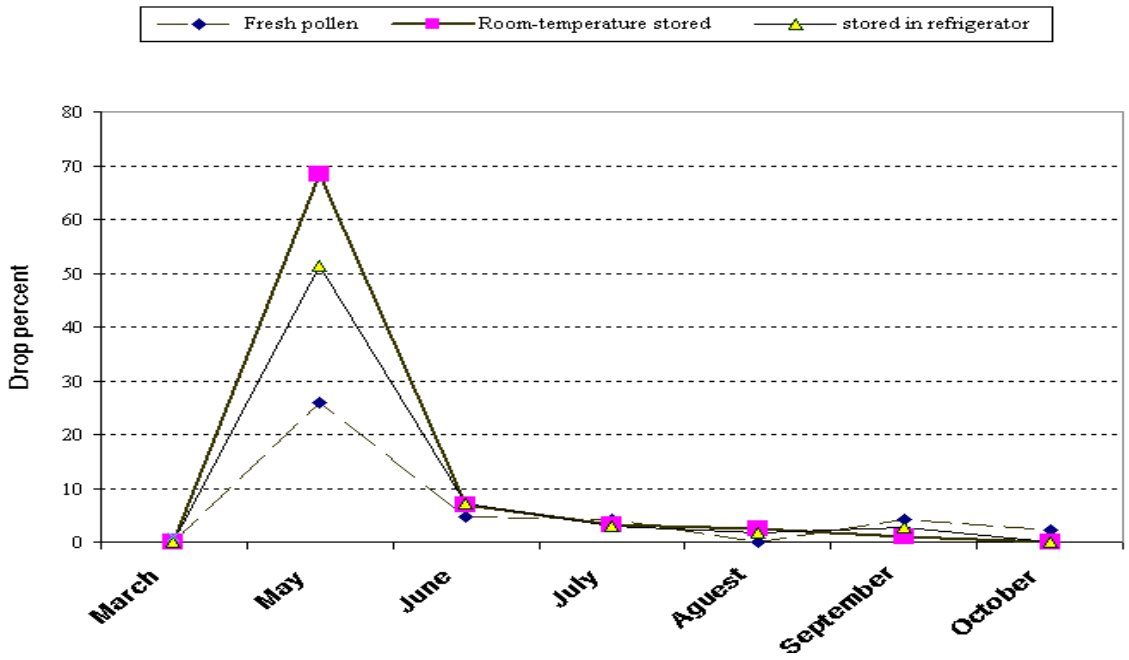

Fig. 2. Drop rate of fruits after pollination through harvest time.

Total Phenolics and Antioxidant Properties of Date Palm (*Phoenix dactylifera* L.) Pits as Affected by Cultivar and Location

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Keywords: date palm cultivars, date pits, ABTS, DPPH, antioxidant, total phenolics.

Abstract

Pits of date palm (*Phoenix dactylifera* L.) fruit constitute about 10 to 15% of the fruit weight and contain fats, carbohydrates, minerals, proteins, steroids, vitamins, phenols, and crude fibers. Although of potentially useful nutritive content, pits generally have had no specific use and are commonly discarded, other than sometimes they may be used as organic fertilizers, as feed supplement for livestock and activated charcoal. There is also an increasing interest in using date pits to produce a caffeine-free hot beverage similar to coffee. Pits of 15 cultivars were collected from the USA and Saudi Arabia in 2006 and 2007 to characterize and compare total phenolics (TP) content and antioxidant capacity and to study the difference between ABTS [-2,2' Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium Salt ~98%] and DPPH (2,2-Diphenyl-1-picrylhydrazyl) methods in measuring antioxidant capacity. Pits of the cultivars collected from Saudi Arabia had higher dry matter content than those collected from the USA. In most of the tested cultivars, total phenolics content was significantly higher during the first year of the study. Pits of 'US6' cultivar ('Hilali') had the highest total phenolics content (66.7 mg GAE/100 g DW) during the first year of the study followed by 'SA3' cultivar ('Deglet Noor') which had a total phenolics content of 66.4 mg GAE/100 g DW. The lowest content was found in the pits of 'US1' cultivar ('Amir Hajj') (14.5 mg GAE/100 g DW) followed by 'US5' cultivar ('Hayany') (16.4 mg GAE/100 g DW) and 'US2' cultivar ('Barhee') (24.6 mg GAE/100 g DW respectively). The pits of 'US3' ('Deglet Noor'), 'US6' ('Hilali'), 'US10' ('Zahidi') and 'SA3' ('Sukari') cultivars had higher antioxidant capacity as measured by both ABTS and DPPH during the first year as compared to the second year of the study while the pits of 'US7' ('Khadrawy') and 'SA1' ('Amir Hajj') cultivars had higher antioxidant capacity during the second year of the study. Mean separation tests indicated that first year samples had higher antioxidant capacity than those of the second year as measured by either ABTS or DPPH. ABTS ranged from 53.1 to 679.0 µmole TEAC/100 g of dry weight in 'US5' ('Hayany') and 'US6' ('Hilali') respectively. Although ABTS analysis had a higher antioxidant capacity as related to the total phenolics contents in most cultivars, DPPH analysis had higher reproducibility. Over all cultivars tested from both years, the antioxidant capacity as related to total phenolics content was higher in samples collected from Saudi Arabia. The pits of different date palm cultivars have different physical and chemical characteristics including dry matter content, total phenolics content and antioxidant capacity. The total phenolics assay may be a good indicator of antioxidant properties in date pits.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated plants on earth. Over the past three centuries, it has been particularly important in the Arabian Peninsula of the Middle East, as well as in southern Africa, Australia, Mexico, and the United States, in southern California, Arizona, and Texas (Chao and Krueger, 2007).

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Fruits of date palm are commonly consumed and are a vital component of the diet in many parts of the world (Vayalil, 2002). The pits of date palm (*Phoenix dactylifera* L.) fruits constitute about 10 to 15% of the fruit weight (Almana and Mahmoud, 1994; Hussein et al., 1998) and contain about 9.0% fat, of which 56.1% is oleic acid, 11.6% linoeic acid, 8.3% lauric acid, 6.0% myristic acid and 2.6% stearic acid (Al-Hooti et al., 1998). Phytochemical and chromatographic screening have documented other compounds in date pits including carbohydrates and minerals, as well as proteins, steroids, vitamins, phenols, and crude fiber (Al-Showiman, 1990; Besbes et al., 2004a,b; Ghazanfari et al., 2008; Hamada et al., 2002). Although of high nutritive value the pits generally have no specific use and are commonly discarded, unless sometimes used as organic fertilizers, feed supplements for livestock and activated carbon as an adsorbent (Aldhaheri et al., 2004; Banat et al., 2003, 2004; Girgis and El-Hendawy, 2002; Hamada et al., 2002).

There is an increasing interest in using date pits in producing a hot beverage similar to processing of coffee beans without the disadvantage of caffeine (Ali-Mohamed and Khamis, 2004; Banat et al., 2004; Barreveld, 1993; Hamada et al., 2002; Haynes and McLaughlin, 2000).

Modern technology and scientific research have demonstrated the importance of food bioactive components in protecting against various illnesses such as cancer and cardiac disease (Al-Farsi et al., 2005; Saways et al., 1982). The interest in antioxidants has been increasing because of their ability to scavenge free radicals associated with various diseases (Silva et al., 2007). Results of in vitro antioxidant assays may not only explain the high oxidative stability of date pits, but could also help to improve the economic utility of date pits, as well as provide data for more extensive investigations into their ability to inhibit in vivo free-radical-mediated damage.

While there is no one recognized analytical standard or preferred method to measure the antioxidant capacities of food (Wu et al., 2004), most researchers agree that several assays are preferred over one. Assays that measure ability to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ are among the most widely and commonly used spectrophotometric methods for determining the antioxidant capacity of foods and chemical compounds (Awika et al., 2003; Kim et al., 2002). Phenolic antioxidants also differ from simple to very highly polymerized (Shahidi and Naczk, 2003).

To the best of our knowledge, in vivo studies on the antioxidant properties of date pits are lacking as is in vitro information on the relative antioxidant status of the pits from the major date palm cultivars grown in the United States of America and Saudi Arabia. The objectives of this study, therefore, were to: 1) characterize and compare total phenolics content and antioxidant activities (free-radical-scavenging activity) of date palm pits among ten cultivars collected from the US and five collected from Saudi Arabia; 2) compare ABTS and DPPH methods in measuring antioxidant activity and test the association of each with total phenolic content in date pits; and 3) determine if production year and location impact antioxidant activity and total phenolics content.

MATERIALS AND METHODS

Materials

Mature fruits of ten cultivars were provided by the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates - Riverside, California, USA and five were provided by Saudi Arabian farmers from authenticated date palms (Table 1). Fruits (2006 and 2007 harvests) were used at full ripeness. Upon arrival at the laboratory, date fruits were hand pitted to separate the pits, and samples were stored at -20°C for extraction and analysis.

Dry Matter Determination

Three replicates of each cultivar were used for the determination of dry matter content (DM %). Pits of each replicate were cleaned by hand of any adhering date flesh, and each pit was split into four fractions. The fresh weights of pit fractions were recorded

and dried at 70°C under vacuum (100 mmHg) to a constant weight to calculate DM %.

Sample Extractions

To determine the best extraction method a pilot study was done using two cultivars ('US8' and 'SA3') and a commercial date pit powder as a standard (produced in 2005 by Al Saad Dates Factory, Emirates). Pits of 'US8' and 'SA3' cultivars were cooked in an oven at 260°C, for 0, 5, 10, 15, 20 or 30 min. Roasted pits were ground with a coffee grinder. Raw unroasted pits are much harder and were ground in a heavy-duty grinder and passed through a 1-2 mm screen. To determine the best cooking/roasting time, extracts were prepared using 80% acetone + 1% formic acid solution. Hydrolysis of samples was performed to liberate bound phenolic acids (Naczk and Shahidi, 2004, 2006; Shahidi and Naczk, 2003). Pits were extracted in methanol containing 1.2 N HCl (40 ml of methanol + 10 ml of 6.0 N HCl) according to Yi et al. (2005). Samples were dissolved in the acidified methanol solution in glass flasks and placed in a water bath at 80°C while shaking at 200 rpm for 2.0 h. The hydrolyzed samples were cooled in an ice bath in the dark and filtered through a 0.2 µm syringe nylon filter.

To determine the best extraction time, 100 mg samples were tested at 1, 3, 6, and 24 h at 4°C. The acid hydrolysis extraction solutions used were: (80% acetone: 19.9% DI water: 0.1% HCl), (80% acetone: 19.9% DI water: 0.1% HNO_3); (70% acetone: 29.5% DI water: 0.5% acetic acid) and (80% acetone: 20% DI water as a non-acidified control). After extraction, all samples were centrifuged at 6000 rpm for 15 min at 4°C. 1 ml of supernatant from each sample was vacufuged to dryness at 45°C for 2 h using a Vacufuge TM, "Eppendorff" and stored at -80°C for further analysis. Three replicates for each cultivar were tested.

Roasting for 10 min, followed by extraction for 1 h in 80% acetone without acid hydrolysis in a water bath (45°C) produced the highest total phenolic content in both cultivars and the commercial powder (Table 2). Accordingly, this protocol was used thereafter for all samples.

Total Phenolic Content

Total phenolic content was determined using Folin-Ciocalteu reagent adapted from Spanos and Wrolstad (1990) and based on the original method of Singleton and Rossi (1965) as slightly modified by Wilson (2003). This assay is based on the color reaction of phenolics with a phosphomolybdic-phosphotungstic acid reagent (Folin-Ciocalteu reagent, Fluka). 35 µl of diluted samples were pipetted per cell in triplicate in a 96 well microplate. With multichannel pipettors, 150 µl of Folin-Ciocalteu reagent (fresh dilute full strength SIGMA reagent diluted 1/10 with DI H_2O) was added into each well. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 s, then held for 5 min at room temperature. After 5 min, 115 µl of 7.5% Na_2CO_3 was added to all wells. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 s. The microplate was placed on a heating pad set to 45°C for 30 min and covered with an insulating styrofoam cover. After standing to cool for 60 min at room temperature, absorbance was read at 765 nm wavelength using a spectrophotometer/microplate reader (SPECTRA *Max*-Plus^{384}, Molecular devices, Sunnyvale, CA) with UV-vis spectral scanning, computer-controlled SOFT *Max* PRO data analysis and reporting software. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of the pit fresh weight (mg GAE/100 g FW). Absorbance readings were compared to control and gallic acid (3,4,5-trihydroxybenzoic acid), which was prepared daily for the generation of a standard curve.

ABTS Radical Scavenging Activity

Antioxidant capacity was measured by -2,2' Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium Salt ~98% (Sigma Aldrich Co.) ABTS assay using the modified method of Miller and Rice-Evans (1997), based on the original method by Miller and Rice-Evans (1996) as slightly modified by Wilson (2003). This procedure

measures the relative ability of antioxidant substances to scavenge the $ABTS^{\bullet+}$ free radical. Oxidation of ABTS by manganese dioxide (MnO_2) (Sigma Aldrich Co.) to the activated $ABTS^{\bullet+}$ radical, is compared with standard amounts of the synthetic antioxidant Trolox (6-Hydroxy-2, 5,7,8-tetramethyl-chroman-2-carboxylic acid ~97% (Sigma Aldrich Chemical Co.), water-soluble vitamin E analogue. This technique is based on the reduction of the blue-green $ABTS^{\bullet+}$ radical by electron- or hydrogen-donating antioxidants, which was estimated by spectrophotometric/microplate analysis (SPECTRA *max*-Plus^{384}, Molecular Devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as µmol Trolox equivalent antioxidant capacity (TEAC) per 100 g of pit dry weight. A stock solution of ABTS was prepared by mixing 5.0 mM ABTS solution with 1-3 g of MnO_2 (manganese dioxide, SIGMA # M-1656) for 20 min at room temperature. The solution was filtered through Whatman® (No.1) filter paper in a Buchner-Funnel using a vacuum pump (DUO-Seal). Excess MnO_2 was filtered by passing the solution through a 0.2 µM syringe filter. This solution was diluted as needed with 5.0 mM phosphate buffered saline (PBS) solution at pH 7.4 and mixed well to read an absorbance of exactly 0.70 (±0.02) at 734 nm. The solution was placed in a controlled water bath at 30°C during use. Fresh $ABTS^{\bullet+}$ radical cation solution was prepared each working day. Antioxidant vitamin E equivalent standard solution was prepared by diluting 6.26 mg Trolox in a 50.0 ml volumetric flask with 5.0 mM PBS. This solution was vortexed and sonicated to thoroughly dissolve the Trolox. Several dilutions (0, 5, 10, 15, 20, 25 µM) were prepared daily from frozen aliquots of the standard. Fresh standards were prepared every 4 weeks. A temperature-controlled cuvette in the Spectra max-Plus^{384} UV-vis spectrophotometer was set at 30°C. Triplicate readings were taken at 734 nm. Linear regression analysis was used to ensure the linearity of the standard curve. Less than 10% Coefficients of variation was used to control treatment, reading and sample preparation variability. Three different dilutions that fell within the linear range of the standard curve (0.2-0.6 AU) were used. Antioxidant capacity was expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in µmole TEAC/100g sample fresh weight.

DPPH Radical Scavenging Activity

Antioxidant radical scavenging activity was also determined using the free radical, 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) microplate assay with methanol according to Lu and Yeap Foo (2000), based on the original by Brand-Williams et al. (1995). A 0.1 mM stock of DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used. The DPPH stock was adjusted to read 0.90 AU in one microplate test cell by using 100% CH_3OH. A standard curve was made by preparing Trolox dilutions (0, 20, 30, 40, 50, 60, 70, 80, 90 µM) using 1.0 mM Trolox and 5.0 mM PBS in 1.5 ml Eppendorf tubes. Fifteen µl of Trolox standard or well-mixed sample solutions were added in triplicate cells. Then 285 µl DPPH stock at 0.90 AU was added to all wells and mixed using a multichannel pipettor. The microplate was covered, allowed to stand for 3 min, and the decrease in the absorbance was read at 515 nm at exactly 25°C using a spectrophotometer/ microplate reader (SPECTRA *max*-Plus^{384}, Molecular Devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Antioxidant capacity was expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in µmoles TEAC/100 g sample fresh weight.

Data Analysis

Data from two years (2006 and 2007) were subjected to analysis of variance using the GLM procedure based on the general linear model procedure of the statistical Analysis System (SAS Institute, 2005, Cary, NC) to test the effect of years, cultivars and their interactions on dry matter content, total phenolics content and antioxidant activity measured by ABTS and DPPH methods. Means separation was performed at $P \leq 0.05$ using Tukey's procedure, Honestly Significant Difference, (HSD). Correlation analyses were done to test the association between total phenolics content and antioxidant activity as measured by both ABTS and DPPH methods. Concordance correlation analysis was

used to assess the agreement between total phenolics content and antioxidant activity over all tested cultivars during the two years of the study. The concordance correlation coefficient (*r*c) provides a measure of reproducibility by evaluating the degree to which pairs of values (*Y*i1, *Y*i2), i=1,2,…,n, depart from a 45° line through the origin (Lin, 1989). The concordance correlation coefficient contains measures of accuracy and precision and examines the strength of a 1:1 linear relationship between X- and Y-axis. Comparisons of concordance correlation coefficients were performed using the Fisher transformation (Zar, 1996) to test the reproducibility of analyses of antioxidant activity comparing ABTS and DPPH methods as related to total phenolics content. The 5% error probability level was used for hypothesis testing throughout. The higher the r_c value the more perfect 1:1 relationship. Location effect was assessed two different ways, first by comparing dry matter content, total phenolics content and antioxidant activity of 'Khalasa' cultivar that was collected from both locations using the GLM procedure based on the general linear model procedure of the Statistical Analysis System. Second, by comparing the antioxidant activity as related to total phenolics content regardless of the cultivars using analysis of variance on the slopes of antioxidant activity versus total phenolics content and locations were compared with regression covariance analysis.

RESULTS

Dry Matter Content

Dry matter content varied significantly among cultivars, between years, and their interactions (Table 3). Dry matter content was significantly higher in the second year samples than those of the first year in some cultivars including 'US1', 'US4', 'US5', 'SA2', 'SA4' and 'SA5' (data not shown). Mean separation tests indicated that over the two years of the study and over all cultivars, 'US5' had the highest dry matter content (92.8%) during the second year of the study while 'US6' had the lowest DM during the first year (84.1.0%).

Total Phenolics (TP) Content

Total phenolics content varied significantly among cultivars and between the 2 years of the study (Table 3). TP content was significantly higher during the first year of the study in 'US3', 'US6', 'US7', 'US9', 'SA3' and 'SA4' while it was significantly higher during the second year of the study in 'US8' and 'SA1' (Fig. 1). Even the pilot study to determine the best extraction method using two cultivars ('US8' and 'SA3') indicated a significant difference among the two cultivars (Table 2). Ranking for TP content indicated that the pits of 'US6' (66.7 mg GAE/100 g DW) and 'SA3' (66.4 mg GAE/100 g DW) had considerably higher TP, (4.6-fold) than the lowest cultivar in the pits of 'US1' (14.5 mg GAE/100 g DW), 'US5' (16.4 mg GAE/100 g DW) and 'US2' (24.6 mg GAE/100 g DW) (Fig. 1).

Antioxidant Capacity

Large and significant differences were detected in antioxidant activity among cultivars as measured by ABTS and DPPH for both years of the study (Table 2). Differences among years were also significant. The pits of 'US3', 'US6', 'US10' and 'SA3' had higher antioxidant activity as measured by both ABTS and DPPH during the first year while the pits of 'US7' and 'SA1' cultivars had higher DPPH antioxidant activity during only the second year of the study (Fig. 2). The pilot study to determine the best extraction method using two cultivars ('US8' and 'SA3') and a commercial date pit powder as a standard (produced in 2005 by Al Saad Dates Factory, Emirates) also indicated a significant difference among cultivars and the commercial powder in antioxidant capacity (Table 2).

First year samples had higher antioxidant activity than those of the second year as measured by either ABTS or DPPH. ABTS values ranged from 53.1 to 679.0 µmole TEAC/100 g of dry weight in 'US5' and 'US6' respectively. In general, pits of 'US6',

'US10', and 'SA3' had the highest antioxidant activity while 'US5', 'US1', 'US2' and 'US4' had the lowest antioxidant activity over all cultivars (Fig. 2).

The Difference between ABTS and DPPH

Both ABTS and DPPH spectrophotometric methods are frequently reported to estimate antioxidant capacity and are usually expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Our results indicated a significant difference between the two methods in quantifying antioxidant capacity. Mean separation tests indicated that antioxidant capacity measured by ABTS was higher than that measured by DPPH in the pits of all cultivars during the two years of the study (Fig. 2). Correlation analysis indicated a significant positive association between total phenolics content and antioxidant activities measured by both ABTS and DPPH analyses (Fig. 3). Although ABTS analysis had higher antioxidant capacity as related to the total phenolics content in most cultivars, concordance correlation analysis indicated that DPPH analysis had higher reproducibility. The agreement between total phenolics content and antioxidant capacity over all tested cultivars during the two years of the study was higher. Comparisons of concordance correlation coefficients to test the reproducibility of analyses of antioxidant capacity comparing ABTS and DPPH methods as related to total phenolics content are summarized in Figure 4. The higher the r_c value the more perfect 1:1 relationship.

Location Effect

While significant differences for dry matter, TP ABTS and DPPH over the two years of the study were detected in the pits of 'Khalasa' ('SA1') cultivar collected from Saudi Arabia compared to 'Khalasa' ('US8') collected from the USA (Fig. 4), the largest differences detected were for ABTS. During both years, the antioxidant capacity of all cultivars as related to total phenolics content was higher in samples collected from Saudi Arabia indicating a higher efficiency of their phenolics content in free-radical-scavenging activity (Fig. 5).

DISCUSSION

Because of the chemical nature of phenolics and other antioxidants that differ from simple to very highly complex (Shahidi and Naczk, 2003), no single solvent extraction method or protocol may be ideal. Accordingly, we tested different protocols to assess the most suitable method for our study. In agreement with Shahidi and Naczk (2003), roasting time of 10 min and extraction time of 1 h in 80% acetone solution were most effective without hydrolysis or alkaline digestion.

Dry matter content varied most among cultivars and less between years. Significant interactions between years of the study and cultivars can be related to cultivar specific inherited characteristics or location conditions including growing condition and soil type, as well as climatic factors which can change from year to year. Due to limitations of the samples available, especially comparing sample locations, it was not possible to identify with certainty the environmental factors that may have contributed to these variations. Fertilizer practices, disease and pest exposure, processing, storage conditions and handling may also be responsible for the observed differences of date fruits (Al-Farsi et al., 2007b; Biglari et al., 2008; Gil et al., 2002; Wu et al., 2006).

The differences in content of total phenolic compounds between locations, years, among cultivars and the years × cultivars interactions can be related to the interaction of a variety of factors such as cultivar and geographic origin, whether it is native or introduced, growing condition, maturity and processing, climatic factors, agricultural practices, diseases and pests, storage conditions, handling and amount of sunlight received (Al-Farsi et al., 2007b; Biglari et al., 2008; Gil et al., 2002). These factors can significantly affect biochemical processes during fruit ripening and as a result affect the content of secondary metabolites (Wu et al., 2006). Although the amount of total phenolics detected in the pits was small compared to that detected in the fruits (Wu et al., 2004; Al-Farsi et al., 2005a, 2007a; Mansouri et al., 2005; Al-Turki, 2008), the pits of

'US6' and 'SA3' were relatively rich in total phenolics and high in radical scavenging capacity (Fig. 3) and could be recommended as sources for date pits rich in antioxidants.

Differences in antioxidant activity as measured by ABTS or DPPH between the two years of the study, among cultivars and their interactions in addition to the superiority of the first year samples to those of the second year for the same cultivar could be related to similar factors that might control dry matter and phenolics content. The fruits of 'US3' that make up about 90% of California's date crop (Hong et al., 2006), were found to be the highest in antioxidant activity (Al-Turki, 2008), while the pits from 'US6' were found to have the highest total phenolics and antioxidant activity indicating that accumulation of total phenolics in date palm fruits and the pits of same fruits may be independent.

The strong correlation detected between total phenolics content and antioxidant activity as measured by ABTS or DPPH suggests that the simpler total phenolics assay may be sufficient to evaluate antioxidant properties in date pits. The total phenolics assay is quicker and less costly than more complicated radical scavenging assays. Several studies have reported positive correlations between antioxidant capacity and polyphenol content (Holasova, 2002; Leontowicz et al., 2003; Javanmardi et al., 2003), while Yu et al. (2002) indicated that no significant associations were found between the total phenolic content and radical scavenging capacity in cereal samples. Kahkonen et al. (1999) pointed out that this might be because different phenolic compounds have different responses in the Folin-Ciocalteu method, or it might be that not all of the total phenolic contents are active radical scavengers or have the same matrix effect (Stushnoff et al., 2003).

ABTS and DPPH are among the most commonly reported spectrophotometric methods widely used in antioxidant activity measurement (Awika et al., 2003; Kim et al., 2002). Currently, there has been no agreement on one preferred method over others in measuring antioxidant capacities of food materials (Wu et al., 2004). The differences in the antioxidant capacity estimation among different assays may be due to the different mechanisms of actions or the difference in reaction conditions. Huang et al. (2005) mentioned that while the DPPH assay is simple, some disadvantages limit its application. In addition, DPPH is a long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH. Consequently, the antioxidant capacity will not be properly rated. Also, it was reported that the reaction of DPPH with eugenol is reversible (Bond et al., 1997). This could result in falsely low readings for antioxidant capacity of samples containing eugenol and other phenols bearing a similar structure type (*o*-methoxyphenol). On the basis of the kinetic analysis of the reaction between phenols and DPPH, Foti et al. (2004) found that the rate-determining step for this reaction consists of a fast electron-transfer process from the phenoxide anions to DPPH. The hydrogen atom abstraction from the neutral ArOH by DPPH becomes a marginal reaction path, because it occurs very slowly in strong hydrogen-bond-accepting solvents, such as methanol and ethanol. In addition, adventitious acids or bases present in the solvent may dramatically influence the ionization equilibrium of phenols and cause a reduction or an enhancement, respectively, of the measured rate constants (Foti et al., 2004). This indicates that the DPPH assay is not necessarily chemically sound as a valid assay for antiradical activity of measurement. We have no information on the nature of possible confounding endogenous compounds in date, thus it is difficult to speculate on the exact nature of the much lower DPPH ratings in this study. Further studies are needed to define the phenolic content of date palm pits to more clearly answer this question.

Although ABTS analysis had higher antioxidant capacity as related to the total phenolics contents in most cultivars, concordance correlation analysis indicated that DPPH analysis had higher reproducibility and the agreement between total phenolics content and antioxidant activity over all tested cultivars during the two years of the study was higher. In other words, the 1:1 linear relationship between total phenolics contents and antioxidant activity as measured by DPPH was higher over all tested cultivars during the two years of the study. The higher concordance correlation coefficient indicated a

stronger 1:1 relationship. A perfect 1:1 relationship helps in developing more accurate regression equations to predict antioxidant activity knowing total phenolics.

Location effect was evident in this study. The slopes of TP against antioxidant capacity as measured by ABTS or DPPH were significantly different (P<0.0001) between the two locations in both years, indicating that as TP increased, Saudi Arabia samples maintained a higher antioxidant capacity as compared to USA samples (Fig. 5). The superiority of samples collected from Saudi Arabia compared to samples collected from the USA was indicated in two different ways. The higher antioxidant capacity as related to total phenolics content in samples collected from Saudi Arabia suggests higher efficiency of their phenolics in free-radical-scavenging capacity. Location effect can be related to the interaction of a variety of factors such as cultivar geographic origin i.e., native or introduced, growing conditions, climatic factors, and amount of sunlight received (Al-Farsi et al., 2007b; Biglari et al., 2008; Gil et al., 2002). These factors can significantly affect biochemical processes during fruit ripening and as a result affect the content of secondary metabolites (Wu et al., 2006). There is no available information regarding location effects on total phenolics and antioxidant capacity of date pits. Many other crops are known to be significantly affected by location (Emmons and Peterson, 2001; Howard et al., 2003; Peterson and Qureshi, 1993). 'Khalasa' ('US8' and 'SA1') is commonly grown in the USA ('US8') and in Saudi Arabia ('SA1'). 'US8' (PI #8753, Accession #78-27) was originally introduced to the USA from Al-Hofuf city (Krueger, 1998; Nixon, 1950) which is the major urban center in the huge Al-Ahsa Oasis in the Eastern Province of Saudi Arabia. Al-Hofuf (25°22'N; 49°34'E) is 10 m a.s.l. and receives less than 100 mm rain per annum. Summers are hot with temperature average exceeding 45°C, and winters are cool with temperature ranging from 14 to 23°C. 'US8' is grown in Riverside CA (33°953'N; 117°395'W) at an elevation of 258 m. a.s.l. with an average annual rainfall of 254 mm. At Riverside, CA the climate is warm during summer when temperatures tend to be in the 30s°C and cool during winter when temperatures tend to be in the 10s°C. The warmest month of the year is August with an average maximum temperature of 35°C, while the coldest month of the year is December with an average minimum temperature of 5°C. These climatic variations in addition to the variation in the altitude between the two locations could affect the physical and chemical characteristics of date fruits. Chatty and Tissaoui (1997) reported that different temperature demands of palm species are related to their sub-tropical origin and their geographic distribution. Temperature would likely influence fruit chemical content depending on the region in which they are grown. Morton (1987) pointed out that heating during ripening of date varies between 25-30°C depending on cultivar and location.

In conclusion, findings from this study indicate that the cultivars as a source of pits contributed the most important source of variation to dry matter, TP and antioxidant radical scavenging capacity compared to variation due to production year or location. Measurement of total phenolics may provide a sufficient indication to evaluate antioxidant properties in date pits. Antioxidant activities measured by ABTS were higher in quantity than those measured by DPPH. While DPPH had higher concordance correlation coefficients that indicate a more perfect 1:1 relationship the low values obtained compared to ABTS as a scavenging assay, suggest important limitations in use of DPPH as a measure of antioxidant properties in date pits. Overall, samples collected from Saudi Arabia had higher antioxidant properties compared to samples collected from the USA. The higher antioxidant activity as related to total phenolics content in samples collected from Saudi Arabia indicates the higher efficiency of their phenolics contents in free-radical-scavenging activity. Further studies are needed to characterize antioxidant properties of date pits for use as a hot, caffeine-free beverage similar to coffee.

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Tables

Table 1. Date palm (*Phoenix dactylifera* L.) cultivars and the abbreviation used throughout the manuscript. Cultivars were collected from USA (location 1) and Saudi Arabia (location 2).

No.	Cultivars	Source	Abbreviation used
1	Amir Hajj	location 1	US1
2	Barhee	location 1	US2
3	Deglet Noor	location 1	US3
4	Halawy	location 1	US4
5	Hayany	location 1	US5
6	Hilali	location 1	US6
7	Khadrawy	location 1	US7
8	Khalasa*	location 1	US8
9	Medjool	location 1	US9
10	Zahidi	location 1	US10
11	Khalasa*	location 2	SA1
12	Shaishi	location 2	SA2
13	Sukari	location 2	SA3
14	Gur	location 2	SA4
15	Khunizi	location 2	SA5

* Same cultivar was collected from both locations.

Table 2. Total phenolics and ABTS antioxidant of pits extracted for 1 h in 80% acetone compared to 100 g date pit powder used as a hot beverage.

	Total phenolics (mg GAE/100 g DW)	ABTS (μmole TEAC/100 g DW)
Cultivar		
Sukari (SA3)	6149.9a*	15754.5a
Khalasa (US8)	4711.7b	14155.0b
Powder**	3658.5c	11212.8c

* Different letters within the same column indicate a significant difference at $P \leq 0.05$.

** Produced in 2005 by Al Saad Dates Factory, Emirates.

Table 3. Analysis of variance with mean squares and treatment significance of date palm pit dry weight, total phenolics, ABTS and DPPH as affected by the two years of the study, different cultivars and their interactions.

Parameter	Source	df	Mean squares	P-value*
Dry weight	Year (Y)	1	286.3	<0.0001
	Cultivar (C)	14	69.0	<0.0001
	Replications	2	14.4	0.052
	Y×C	14	40.5	<0.0001
Total phenolics	Year (Y)	1	338225	<0.0001
	Cultivar (C)	14	72236	<0.0001
	Replications	8	1864	0.14
	Y×C	14	10828	<0.0001
ABTS	Year (Y)	1	306772	<0.0001
	Cultivar (C)	14	2944840	<0.0001
	Replications	26	26964	<0.0001
	Y×C	14	1032035	<0.0001
DPPH	Year (Y)	1	440584	<0.0001
	Cultivar (C)	14	687886	<0.0001
	Replications	8	5499	0.1400
	Y×C	14	79897	<0.0001

* Significant at $P \leq 0.05$.

Table 4. Analysis of co-variance with mean squares and treatment significance of the antioxidant activity as related to total phenolics content and affected by location over all test cultivars during the first and second year of the study.

Year	Source	df	ABTS		DPPH	
			Mean squares	P-value*	Mean squares	P-value
First year	Location	1	3454786	<0.0001	247865	0.0025
	Total phenolics×location	2	951542	<0.0001	2914334	<0.0001
	Slopes	1	3009	<0.0001	514413	<0.0001
	Intercepts	-	-	0.1700	-	<0.0001
Second year	Location	1	1421618	<0.0001	715354	<0.0001
	Total phenolics×location	2	1325862	<0.0001	4484780	<0.0001
	Slopes	1	352446	<0.0001	247377	<0.0001
	Intercepts	-	-	<0.0001	-	0.0002

*Significant at $P \leq 0.05$.

Figures

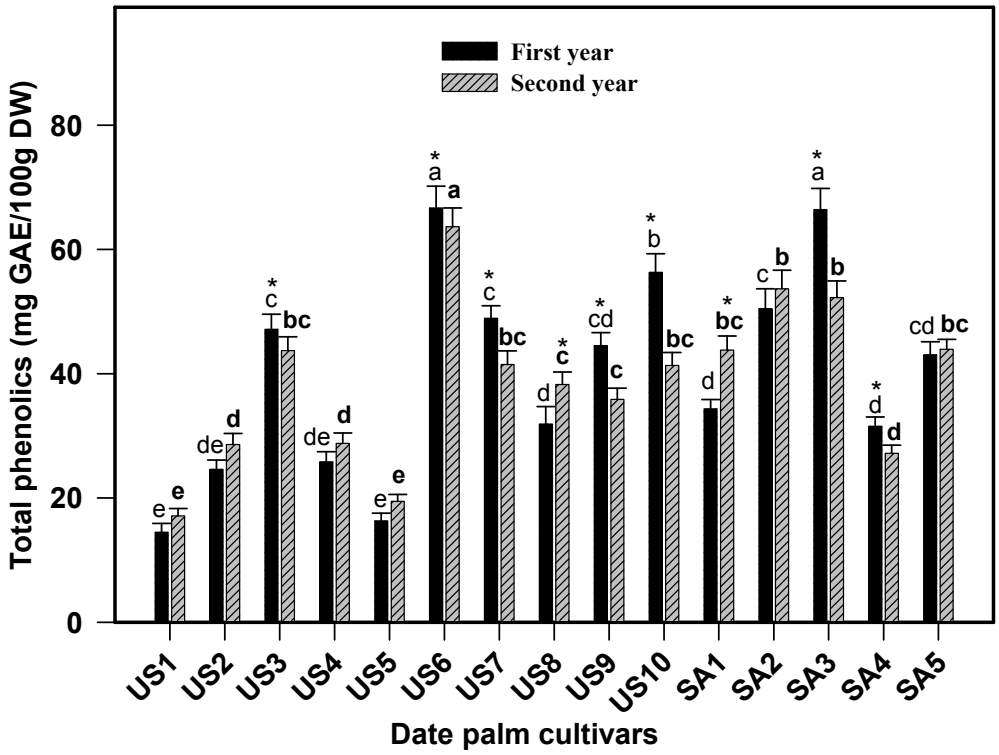

Fig. 1. Total phenolics (TP) content of the pits of the tested date palm cultivars during the first and second years of the study. Columns labeled with the same letter are not significantly different at P=0.05 within the same year. Columns labeled with a (*) are significantly higher (P=0.05) for year comparison within same cultivar. Vertical bars at the top represent standard errors.

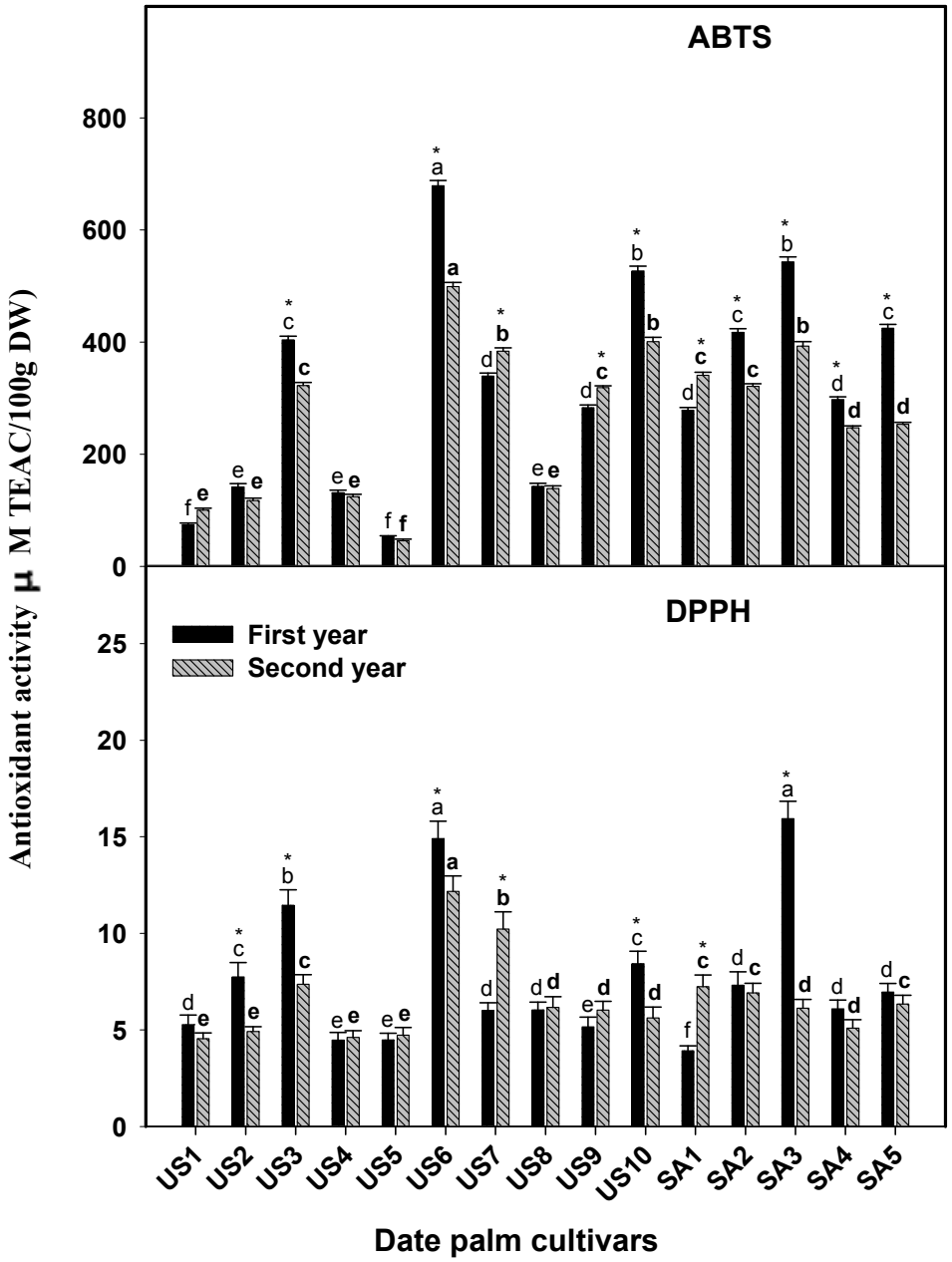

Fig. 2. Comparison between ABTS and DPPH assay data in determining antioxidant capacity (TEAC) in the tested cultivars (See Table 1) during the first and second years of the study. Columns labeled with the same letter are not significantly different at *P*=0.05 within the same year. Columns labeled with a (*) are significantly higher (*P*=0.05) for method comparison among cultivars. Vertical bars at the top represent the standard errors.

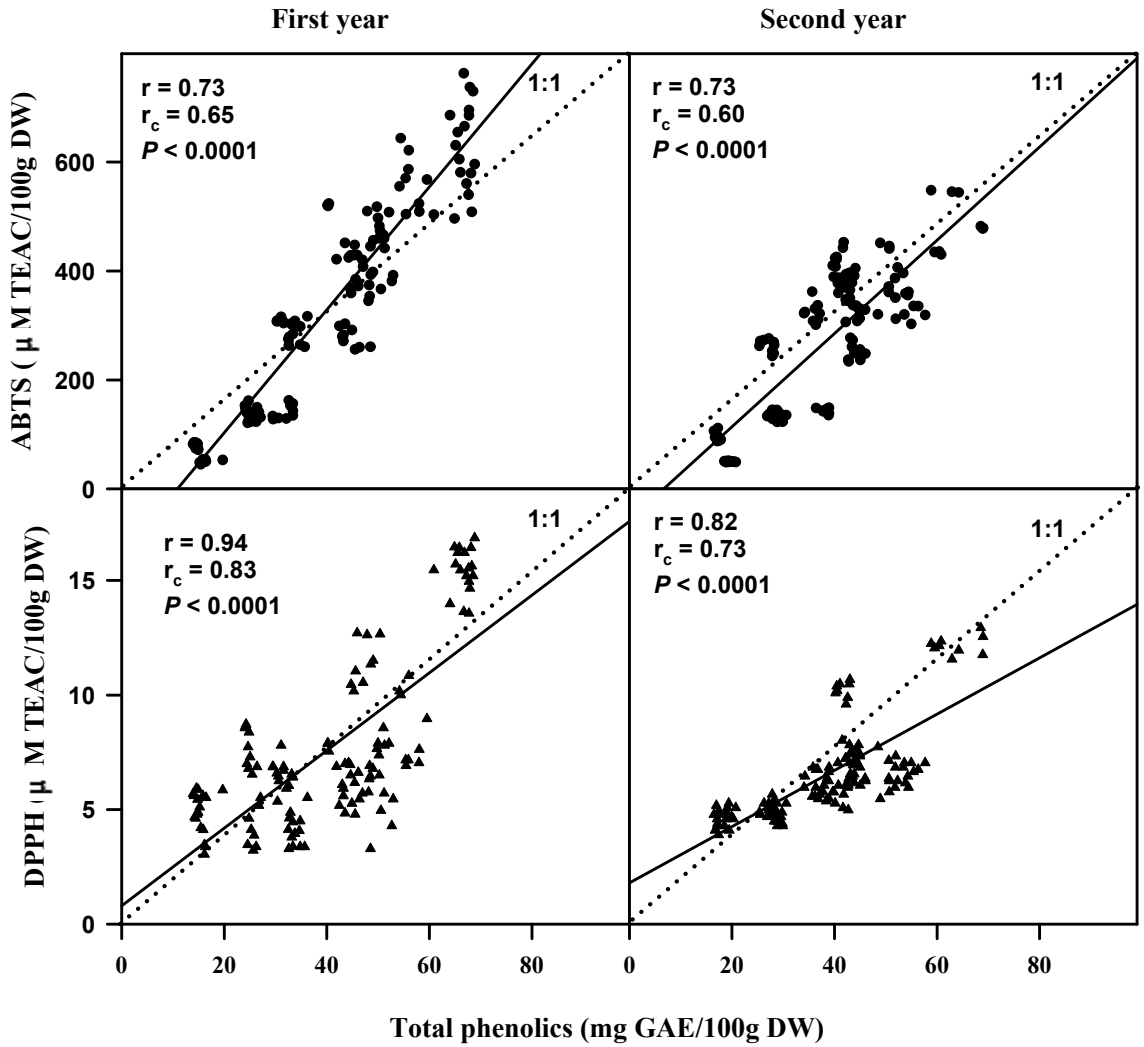

Fig. 3. Reproducibility of analyses of antioxidant capacity as related to total phenolics content comparing ABTS (top) and DPPH (bottom) assays over all tested cultivars during the first year (left panel) and second year (right panel) of the study. Correlation coefficients (r), concordance correlation coefficients (r_c) and their significance are reported in each panel along with a 45° 1:1 line throught the origin (dotted line) that reprsents perfect reproducibility.

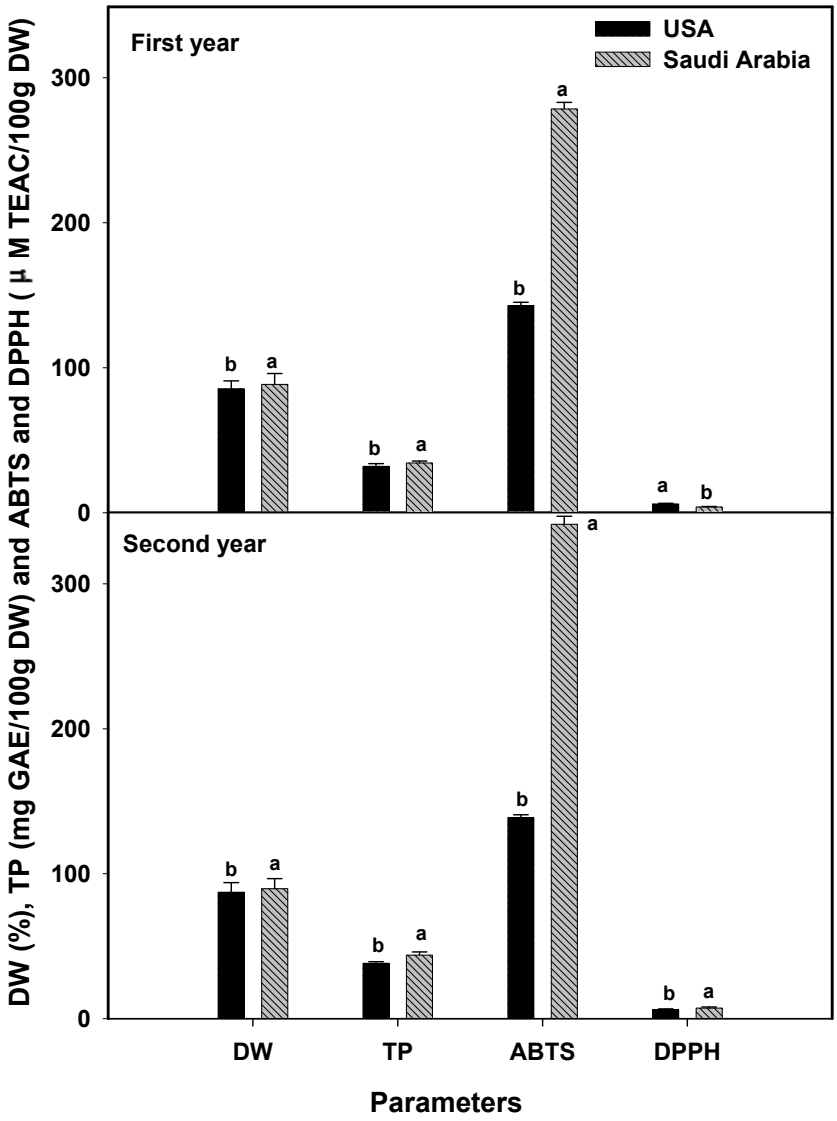

Fig. 4. Effect of location on 'Khalasa' cultivar pits dry matter content percentage (DW), pits total phenolics content (TP) and pits antioxidant capacity (ABTS or DPPH) during the first (top) and second (bottom) years of the study. Columns labelled with the same letter are not significantly different at P=0.05 for location comparison within each parameter. Vertical bars at the top represent the standard errors.

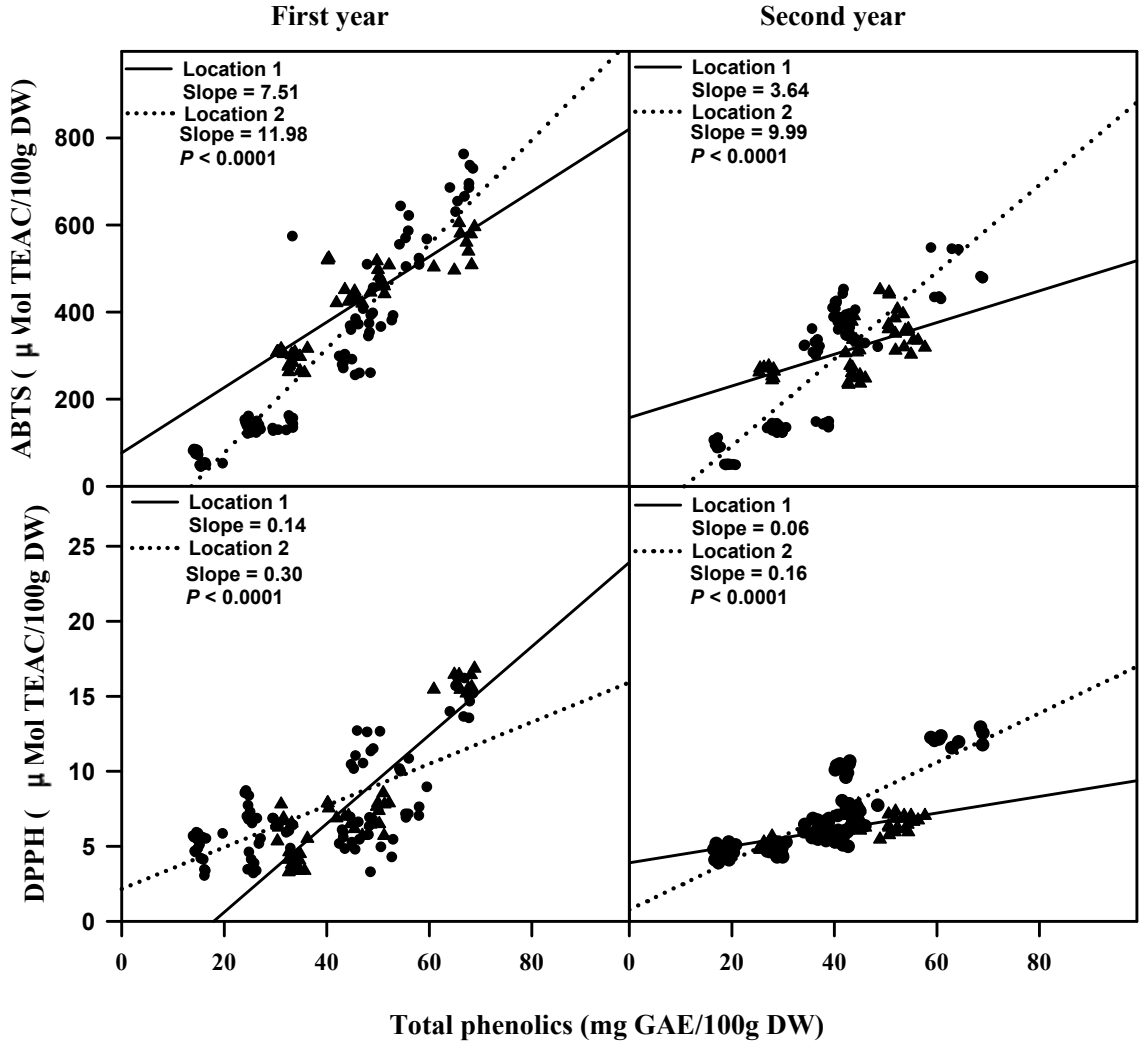

Fig. 5. The effect of location (USA = location 1, Saudi Arabia = location 2) on antioxidant capacity as related to total phenolics content of the pits over all tested cultivars during the first year (left panel) and second year (right panel) of the study. Slope values and co-variance analyses significance are reported in each panel.

Thermal Behavior of Milk Drink Flavored with Date Syrup Measured by MDSC and Predicted by Artificial Neural Networks (ANNs)

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Keywords: MDSC, thermal behavior, dates syrup, milk drink, ANN

Abstract

The thermal behavior of a new drink of milk flavored with concentrated date syrup was evaluated. A modulated differential scanning calorimeter (MDSC) was utilized to study the thermal properties of milk-dibbs drinks at different concentrations for a temperature range of -65 to 65°C. The concentrated date syrup (dibbs) added ranged from 10 to 30 ml/100 ml milk. Onset melting temperature, melting point of fusion, and latent heat of fusion for all concentrations (0-30 ml dibbs/100 ml milk) fell within the ranges -6.03 to -18.94°C, 0.44 to -5.46°C and 275.7 to 164.3 J g^{-1}, respectively.

The apparent specific heat values decreased with the increase of milk-dibbs drink concentration in both phases (liquid and solid). The mean values of apparent specific heat above freezing (10 to 65°C) were 4.16, 3.92, 3.81, 3.70, 3.38 and 2.90 kJ kg^{-1} C^{-1} for 0, 10, 15, 20, 25, 30 ml dibbs/100 ml milk, respectively. At the phase change, the peak areas were higher for the high water content solutions. However, the dibbs heat flow curve obtained did not exhibit phase transition at the studied temperature range, which can be attributed to the high °Brix values (75%) of sugar concentration. The specific heat values of pure dibbs fell within the range 1.48 kJ kg^{-1} C^{-1} (at -65°C) to 4.12 kJ kg^{-1} C^{-1} (at 65°C). It was found that the Artificial Neural Networks (ANNs) technique is a powerful tool to predict the apparent specific heat based on temperature, concentration of milk-dibbs drink, Brix, pH, and water activity.

INTRODUCTION

Dates (*Phoenix dactylifera* L.) are the top produced fruit in Saudi Arabia with an estimated production of 992,000 tons in 2009 (Agriculture Statistical Yearbook, 2010). The dibbs (high concentration syrup) of date fruits have a desiarable natural sweet flavour which makes it an important component that can be utilized in many food industry applications. It has a golden colore, caramel-like flavour and a delicious taste with a sugar cotent of 75°Brix. It can be used as a sweetener and nutritious component in the production of bevarges, carbonated and-non carbonated as well as alternative sweetener in the dairy industry (Hassan and Hobani, 2002). The milk industry is a well developed sector in Saudi Arabia producing more than 1,100 million liters per annum (Agriculture Statistical Yearbook, 2008). Milk drink flavored with date dibbs has a high nutritional value and no artificial additives or preservatives (Alhamdan, 2002). The date syrup (dibbs) is produced via a sequence of unit operations including extraction, filtration, and concentration of the date juice by evaporation under vacuum. Similar to other high sugar fruit juices, the date syrup is a heat-sensitive product which requires a careful understanding of its thermal behavior and quality during different processing stages (Assiry and Elansari, 2003).

Thermal properties data are essential in heat treatments of foods. Specific heat determines the amount of sensible heat that needs to be removed or added from the food

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product. Latent heat of fusion is utilized to determine the heat to be removed at the phase transition during the freezing process. The range values of those thermal properties depend mostly on the composition and thermal phase of the product.

The Differential Scanning Calorimeter (DSC) has been widely used to study various thermal behaviors during the phase transitions of food sugar solutions (Ferrero and Zaritzky, 2000; Roos and Karel, 1991; Roos, 1987). From the phase transition curves, properties such as glass transition (T_g), incipient melting point (T_{im}) and latent heat of fusion can be determined. Traditional DSC, as a thermal analysis technique, has several advantages including fast analysis, easy sample preparation applicabale to both solids and liquids, and wide temperature range. More recently, the Modulated Differential Scanning Calorimeter, (MDSC) overcame some of the limitations of conventional DSC including the compromise between sensitivity and resolution and the requirement for multiple experiments for heat capacity measurements (Leonard, 2001 a,b,c,d; Viciosa et al., 2007).

The Artificial Neural Network (ANN) technique has emerged as a powerful tool that can be used for various scientific and engineering applications such as process control and system modeling. ANNs are inspired by the nervous biological architecture systems consisting of relatively simple systems working in parallel to facilitate quick decisions (Mohammadreza et al., 2009). ANN is formed from hundreds of single units, artificial neurons or processing elements (PE), connected with coefficients (weights), which constitute the neural structure organized in layers. Each PE has weighted inputs, transfer function and one output. Neural network training is achieved when the error function is minimized, thus predicting the targeted formula. There are several types of neural networks that can be classified into transfer functions of neurons, the learning rule, and the connection formula (Eslamloueyan and Khademi, 2009).

ANNs have been used for kinetics of enzyme inactivation (Geeraerd et al., 1998a,b); fermentation (Dornier et al., 1995) and crossflow microfiltration (Latrille et al., 1993). Simulated processes in drying behavior were determined for several agricultural commodities such as carrot (Erenturk and Erenturk, 2007; Kerdpiboon et al., 2006), tomato (Movagharnejad and Nikzad, 2007), ginseng (Martynenko and Yang, 2006) and cassava and mango (Hernandez-Perez et al., 2004; Trelea et al., 1997). Furthermore, ANNs have been implemented to predict thermal conductivity of food products as a function of moisture content, temperature, and apparent porosity (Sablani and Rahma, 2003), thermal food processing operations (Chen and Ramaswamy, 2002), thermal inactivation of pathogenic and spoilage bacteria (Lou and Nakai, 2001), degradation of vitamins during drying processes (Kamiński and Tomczak, 2000), and wine characteristics (Vlassides et al., 2001). Recently, Du and Sun (2006) reviewed the learning techniques used in computer vision for the evaluation of food quality. ANNs have been successfully applied for predicting food quality (Ni and Gunasekaran, 1998; Xie and Xiong, 1999) such as beef sensory (Park et al., 1994), shelf life of pasteurized milk (Vallejo-Cordoba et al., 1995), sensory attributes of noodles (Tulbek et al., 2003), and shelf life of soya milk (Ko et al., 2000). Alhamdan et al. (2001) utilized the ANN technique to predict the apparent viscosity of a milk-dibbs drink. They concluded that the ANN technique can prediect the apparent viscocity of milk-dibbs precisely.

Knowledge of thermal behavior of milk drink flavored with date dibbs is essential for process design, optimization, and quality control and to satisfy consumer's demands. There is a need for the food industry to explore the utilization and processing of this new drink at different milk-dibbs drink concentrations. No data are yet available of the thermal properties of milk drink flavored with date dibbs. Thus, there is a need to measure and provide basic physical and thermal properties of such a new product for pasteurization and sterilization processes.

The purpose of this study was to evalute the thermal behavior of a milk drink flavored with date dibbs at different concentrations in a wide temeprature range (-65 to 65°C) utilizing the MDSC method. The ANNs technique is utilized to predict apparent specific heat of milk-dibbs drink at variable percentage, temperature, total soluble solids, pH and water activity.

MATERIALS AND METHODS

Sample Preparation and Physical Measurements

The preparation of the milk-dibbs drinks was similar to the procedure developed by Alhamdan (2002). Full cow cream sterilized milk was obtained from the local market in a half liter container. Dibbs (concentrated date-syrup from 'Ruziz' date cultivar) was purchased from a local date processing plant. The process of producing dates dibbs consists of three stages: extraction of sugar by mixing added hot water with dates (3:1 of water: dates, w/w), filtration, and concentration under vacuum to 75°Brix (±0.1) date dibbs. To formulate the required milk-dibbs drinks, amounts of 10, 15, 20, 25, and 30 ml of dibbs were added to 100 ml milk for the five mixes used. The amount of dibbs added to the milk-dibbs drinks and their properties are shown in Table 1. The total soluble solids (TSS) content of the mixes was determined by refractometer (Bellingham-Stanley Limited, London, England). Water activity (a_w) of milk-dibbs drinks at different concentrations was measured using AquaLab (Model CX-2, Decagon Devices Inc., Pullman, WA; precision ±0.003) utilizing the chilled-mirror dew point technique. The instrument was calibrated with distilled water (a_w=1.0) (Mohsenin, 1986). The moisture content (MC) was determined using AOAC procedures (AOAC, 1995) where the samples were dried at 70°C for 48 h under a vacuum of 200 mm mercury (Vacutherm model VT 6025, Heraeus Instrument, D-63450, Hanauer, Germany). Finally, pH was measured using a pH meter (WTW model pH528, Germany).

Modulated Differential Scanning Calorimeter (MDSC)

The thermal behavior of milk-dibbs drinks was determined using a thermal analysis system (MDSC Q100, TA Instruments, Ltd., Leatherhead, England). The MDSC is equipped with a two-stage mechanical cooling system and Advantage V1.2.0.147 software. The system heat flow was calibrated with pure indium (melting point 156.4°C and heat fusion 28.45 J/g) while temperature calibration was preformed with Sapphire.

At the start of the experiments, the MDSC cell was flushed with dry nitrogen gas (AGA, 99.9% N_2) at 50-60 ml/min to eliminate water condensation. An empty aluminum pan was used as a reference sample. Triplicate samples of 15-20 mg were hermetically sealed (Sample Encapsulating Press, TA Instruments) in preweighed 40 µl aluminum DSC pans. The samples in the pressed pans were cooled down to -65°C at the maximum cooling rate and then scanned at a heating rate of 2°C/min to 65°C to avoid burning effect. A modulation period of 100 s and a modulation temperature of 1°C were set for all experiments. The heat behavior was plotted as funcution of temperature from -65 to 65°C utilizing Advantage software.

The incipient melting point (T_{im}) of each mix was determined from the heat flow curve (enthalpy versus temperature) in which a clear molten fraction was observed (Ross, 1986). The latent heat of fusion (ΔH_m) was determined by integrating the peaks in the melting curve for the temperature at phase transition region utilizing the horizontal sigmoid baseline. The start and the end points of melting curves were determined using an objective calculation algorithm of the slope of the heat flow curve first. Then, the temperature at which the slope changes from negative to positive before the maximum peak is considered to be the starting point, and the temperature at which the slope changes sign for the second temeprature after the maximum peak is taken as the end point. The area between the start and end points was calcultaed which represents the amount of heat released up to that temeprature. This integration was performed utilizing the Advantage software.

Statistica® version 7 by StatSof Inc. OK, USA, was utilized for statistical analysis with LSD (0.05 significance level).

Artificial Neural Network Construction

The network was developed using Qnet® for windows V2k build 721 (Vesta Services Inc., IL, USA). The network was simulated based on a multi-layer feed-forward

algorithm. The temperature (Temp), dibbs added percentage (ADD), total soluble solid (Brix), pH and water activity (a_w) were used as the inputs, whereas the apparent specific heat (C_p) was the output.

The optimal network architecture is achieved through a trial and error procedure. The structure of the network can be performed through varying the number of hidden layers as well as the number of neurons within each hidden layer. Too few neurons in the hidden layer impairs the network and prevents the network to be trained appropriately. On the other hand, too many nodes allow the network to memorize the pattern (i.e., develop a correlation) presented without capturing the underlying relationship between the input and output variables (Shafafi and Devahastin, 2009).

To select an optimum ANN configuration, the architectures were performed with one and two hidden layers with 4, 6, 8 and 10 nodes in each hidden layer. To achieve the combination of hidden layers and neurons that produced the minimum error, a sigmoid function was used as the transfer function in each hidden layer and was used in the output layer. The optimized configuration based on the training of each neuron was selected from the eight configurations based on the neural network performance, which gave the minimum error from the training process. The average mean square error (MAE), standard deviation (STD), percentage of relative mean square error (MRE%), and standard deviation of MRE% (STDa) were used to compare the performance of different ANN configurations. MRE% and STDa were calculated according to:

$$MRE\% = \left(\frac{1}{N}\sum_{i=1}^{N}\Delta X_a\right)\times 100 \quad (1)$$

$$STD_a = \sqrt{\frac{\sum_{i-1}^{N}(\Delta X_a - \overline{\Delta X_a})^2}{N-1}} \quad (2)$$

where $\overline{\Delta X_a}$=average ΔX_a; N=number of observations; $\Delta X_a = \left|(X_p - X_t)/X_t\right|$, X_t=target value; X_p=predicted value.

To ensure all the variables received equal attention during the training process, it is recommended to rescale the data (Maier and Dandy, 2000). Data were normalized to the interval [-1, 1] based on the following correlation:

$$Y = 2.\frac{X - X_{min}}{X_{max} - X_{min}} - 1 \quad (3)$$

where: X and Y are the original and normalized input or output data, and X_{min} and X_{max} the minimum and maximum values of the input and output data, respectively. In addition, the output data were rescaled into the interval -1 and 1 to allow for the application of the transfer function (log-sigmoid function) in the ANN.

RESULTS AND DISCUSSION

From the heat flow curves of milk-dibbs drinks in the range of -65 to 65°C the apparent specific heat, latent heat of fusion, onset melting point, and the melting temperature were determined.

Heat Flow Curves

Figure 1 shows the heat flow curves obtained at different milk-dibbs drinks. The increase of added dibbs to the milk-dibbs drink narrowed the area under the curve and subsequently increased the melting point. The heat required of the frozen solutions to thaw was the highest for the milk-dibbs drink of 10%, while milk-dibbs drink of 30% was

the least. It is clear that sugar and moisture content in the milk-dibbs drinks have a major effect on their thermal behavior. This is apparent since frozen pure milk needs a higher amount of heat to thaw while the pure dibbs is the lowest. It is interesting to note that dibbs did not show apparent peak areas (phase transition) in the range of measured temperature. As shown in Figure 1 the line of dibbs implies that there is no apparent freezable water in the temperature range studied. This could be attributed to the super-cooled status of dibbs at this range of temperature rather than in freezable status. This needs further investigation to utilize MDSC annealing technique and examine the molecular structure of such high sugar solutions.

Latent Heat of Fusion

The enthalpy of the endothermic transition (latent heat of fusion, ΔH, kJ kg^{-1}) for different milk-dibbs drinks ranged between 164.3 and 275.7 kJ kg^{-1} (Table 2). The data of the current study are in agreement with Parducci and Duckworth (1972) and Ross (1986) that products with lower moisture content had a lower melting point and broader melting peak. It is apparent that pure water has a higher latent heat of fusion (333 kJ kg^{-1}) compared to that of fresh milk (275.7 kJ kg^{-1}) since the latter composition contains solids of 8.5% non-fatty solids and 3% fat. Latent heat of fusion was plotted versus dibbs added as shown in Figure 2 which was found to be linearly correlated (R^2=0.95).

Melting Temperatures

Onset (initial) melting point (T_{mi}, °C) and melting point temperature (T_m, °C) are listed in Table 2 which were determined from the heat flow curves (Fig. 1). The onset melting points of high concentrated solutions occurred at low temperature. For the 30% milk-dibbs drink, the T_{mi} was -18.94°C while for 10% milk-dibbs drink T_{mi} was -12.08°C. The endothermic transition of the melting point has a broader temperature range similar to other foods which show wide melting ranges (Roos, 1992). Peak temperature or the melting point (T_m, °C) ranged from -5.46 to 0.44°C for the different milk-dibbs drinks.

Apparent Specific Heat

The effect of temperature on the apparent heat capacity is shown in Figure 3. Each curve represents an average of three scans where the maximum difference between repeated scans was less than 0.08 mW, which corresponds to 1.65×10^{-2} kJ/(kg °C). This indicted that all changes detected in MDSC heating curves in the present study were reproducible. It is apparent that the peaks in the heating curves were due to the phase change during the melting process representing the decrystallization of the super-cooled liquid fraction in the milk-dibbs drinks.

It is known that the increase of the solid content of foods causes a decrease of specific heat. This was the case for the region above the freezing point of milk and dibbs-milk drinks. However, this phenomena interestingly did not hold for the temperature region below freezing point where the value for dibbs were in most portion of the curve higher than that for fresh milk which needs further investigation. Viscosity of fresh milk, dibbs, and all milk-dibbs drinks under study were significantly different (Alhamdan et al., 2001) which can be correlated with thermal behavior in further investigation.

The Effect of Added Dibbs on Apparent Specific Heat

1. Below Freezing Point. A simple linear relationship fits adequately in correlating the apparent specific heat with temperature below freezing point for the temperature range -20 to -65°C. The correlation coefficient for the different milk-dibbs drinks ranged from 0.89 to 0.95 (Table 3). The apparent specific heat increased with increasing of temperature, but more prominently at higher temperatures. The effect of milk-dibbs drink concentrations showed that the apparent specific heat decreases with concentration. For example, C_p increased from 1.46 kJ/kg °C (for 30% concentration) to 1.85 kJ/kg °C (for 10% concentration); at -45°C. Keppeler and Arboleda (1981) and Alvardo (1991) observed the same trend for sucrose solutions.

2. Above Freezing Point. Table 4 shows the C_p values above freezing point for different milk-dibbs drinks. The values of C_p were averaged on the temperature range 10 to 65°C for each milk-dibbs drink concentration (STD ranged from 0.037 to 0.011). The mean values of apparent specific heat above freezing were 4.16, 3.92, 3.81, 3.70, 3.38 and 2.90 for milk-dibbs drink concentrate 0, 10, 15, 20, 25, 30%, respectively. These trends are in agreement with the data reported by Kapseu and Kaye (1994) and Swern (1979).

It can be noticed that the average apparent specific heat is significantly different (α=0.05) at 30% milk-dibbs drink compared to other drinks. It is apparent that the more viscous solution at such higher concentration contributes to this significant reduction of C_p values.

The Effect of Temperature on Apparent Specific Heat

Data in Table 5 showed no significant effect (α=0.05) of temperature (in the range 15 to 60°C) on averaged apparent specific heat for all tested milk-dibbs drinks concentrations. On the other hand, the temperatures within the range -20 to -60°C have a significant effect on average C_p. In general, increase in the concentration of milk-dibbs drink decreases the specific heat apparently due to the lower water content and higher sugar content.

ANN Analysis Results

The total apparent specific heat data set (736 cases) was randomly divided into three groups. The first group (486 cases) was used for the training process; the second group (150 cases) was for validation (testing); and the last group (remaining 100 cases) was used to verify the model. The error measures associated with different ANN configurations for testing data set of the apparent specific heat are presented in Table 6.

The best prediction network model was set with two hidden layers and eight in each hidden layer with log-sigmoid transfer function in the hidden layers and in the output layer. The MAE, STD, MRE% and STDa for this configuration were 0.1091, 0.1199, 4.3744 and 0.0430, respectively. The results demonstrated good agreement between the predicted and the experimental data of apparent specific heat for both of training and validate data set as shown in Figure 3.

The performance of the optimal neural network was verified using a smaller data set consisting of 100 cases. This network predicted apparent specific heat with MAE, STD, MRE% and STDa 0.151, 0.1765, 5.158 and 0.0494. Correlation between predicted and experimental apparent specific heat of the ANN model is shown as Figure 4 with R^2=0.9343. In addition to examine the ANN model performance, an average contribution of inputs on output was investigated. The percentages were 37.81, 22.62, 9.39, 19.90, and 10.28 for temperature, milk-dibbs drink concentration, total soluble solids, pH, and water activity; respectively.

CONCLUSION

Modulated DSC was proven to be a powerful and accurate tool to obtain thermal behavior parameters of milk-dibbs drinks in a wide range of temperatures (-65 to 65°C). The apparent specific heat values ranged between 1.3 to 4.16 kJ/kg °C based on temperature and milk-dibbs drink concentration. The increase of milk-dibbs drink concentration decreased the specific heat for both above and below freezing points. The latent heat values were higher for the lowest milk-dibbs drink concentration (high moisture content) and found to be linearly correlated (R^2=0.95). Heating curve of dibbs showed no apparent phase transition due to the high sugar content and lower water content. Thus, it is recommended to expand the temperature range applied in this study to detect such transition.

Moreover, ANN could be used as a powerful and simple technique for prediction of the apparent specific heat without the need of performing experiments, and in simulation of unit operations requiring specific heat values as a function of temperature, concentration, pH, and water activity.

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Tables

Table 1. Physical properties of milk-dibbs drinks*.

Sample	ml dibbs/100 ml milk	MC (%)	TSS (%)	pH	a_w
1	0	88.70	8.5	6.81	0.995
2	10	79.14	27.2	6.57	0.982
3	15	76.75	29.4	6.43	0.980
4	20	73.28	31.8	6.32	0.973
5	25	69.31	35.7	6.24	0.976
6	30	68.39	38.3	6.21	0.963

* Milk-dibbs drink: amount of concentrated date syrup (dibbs) added to milk.
MC (db): moisture content, dry bases; TSS: total soluble solid (g/g solution); pH: the decimal logarithm of the hydrogen ion activity in an aqueous solution; a_w: water activity.

Table 2. Latent heat of fusion for milk-dibbs drinks at different levels of added dibbs.

Dibbs added (ml to 100 ml milk)	T_{mi} (°C)	T_m (°C)	ΔH (J g^{-1})
0	-6.03	0.44	275.7
10	-12.08	-0.36	221.3
15	-15.39	-1.33	198.4
20	-16.81	-3.03	195.8
25	-17.89	-4.01	184.3
30	-18.94	-5.46	164.3

T_{mi} (onset melting point), T_m (melting point) and ΔH (latent heat of fusion).

Table 3. Constants of the linear equation of specific heat below freezing point for milk-dibbs drinks (C_p=a T (°C) + b) at the temperature range - 20 to -65°C.

Dibbs added (ml to 100 ml milk)	a	b	R^2
0	0.0165	2.48	0.95
10	0.0355	3.46	0.89
15	0.0414	3.93	0.90
20	0.0478	4.36	0.91
25	0.0529	4.23	0.90
30	0.0392	3.45	0.92

Table 4. Specific heat values above freezing for milk-dibbs drinks averaged within the temperature range 10 to 65°C.

Dibbs added (ml to 100 ml milk)	a_w	Cp (kJ/kg °C)*	SD
0	0.995	4.16[a]	0.037
10	0.982	3.92[a]	0.035
15	0.980	3.81[a]	0.034
20	0.973	3.70[a]	0.015
25	0.976	3.38[a]	0.012
30	0.963	2.90[b]	0.011

* Values with same letter are not significantly different (α=0.05).

Table 5. The effect of temperature on averaged C_p of milk-dibbs drinks.

Temperature (°C)	C_p (J/g °C)*	Temperature (°C)	C_p (J/g °C)*
15	3.708[a]	-20	3.277[b]
20	3.693[a]	-30	2.485[c]
30	3.689[a]	-40	1.924[d]
40	3.682[a]	-50	1.584[de]
50	3.679[a]	-60	1.399[e]
60	3.667[a]		
65	3.661[a]		

* Values with same letter are not significantly different (α=0.05).

Table 6. Error measures for training data set.

Hidden layer	Nodes	MAE	STD	MRE	STDa
1	04	0.1271	0.1351	5.0565	0.0567
	06	0.1363	0.1446	5.5325	0.0619
	08	0.1321	0.1402	5.1602	0.0498
	10	0.1423	0.1563	5.4827	0.0574
2	04	0.1239	0.1281	4.7795	0.0546
	06	0.1241	0.1285	5.0005	0.0566
	08	0.1091	0.1199	4.3744	0.0430
	10	0.1110	0.1135	4.4541	0.0540

Figures

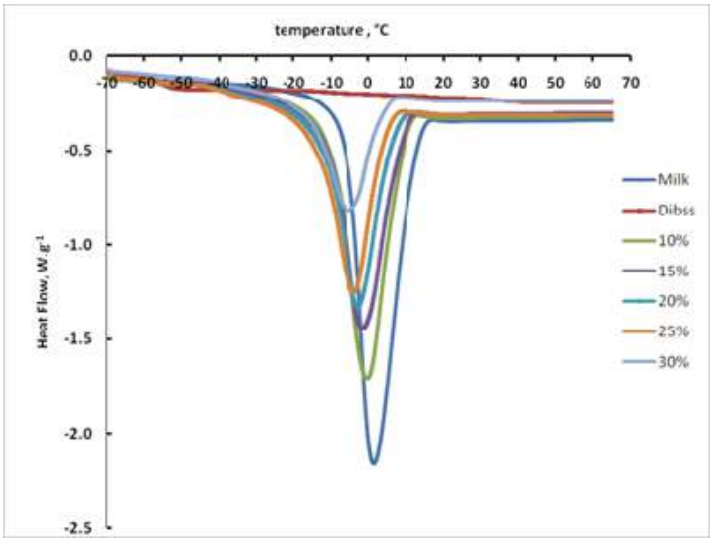


Fig. 1. Heat flow curves for dibbs, milk, and different milk-date extract mixtures.

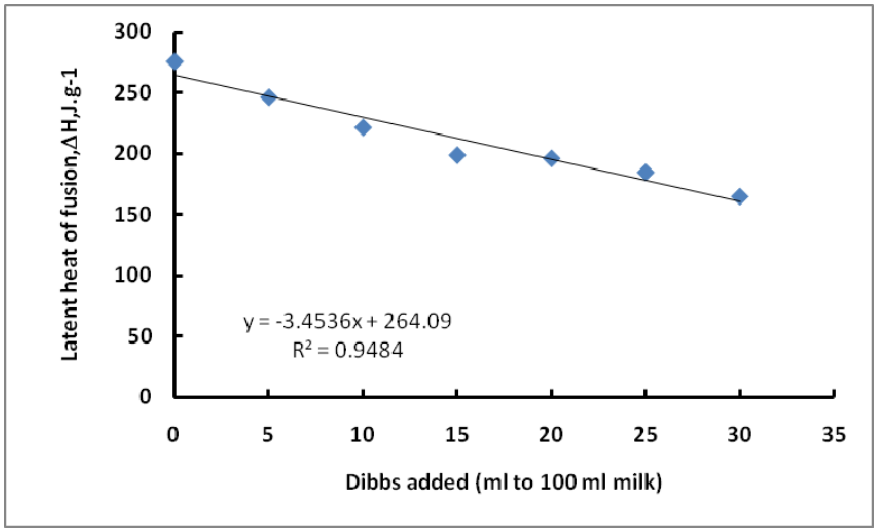


Fig. 2. Latent heat of fusion of milk-dibbs drinks as a function added dibbs concentration.

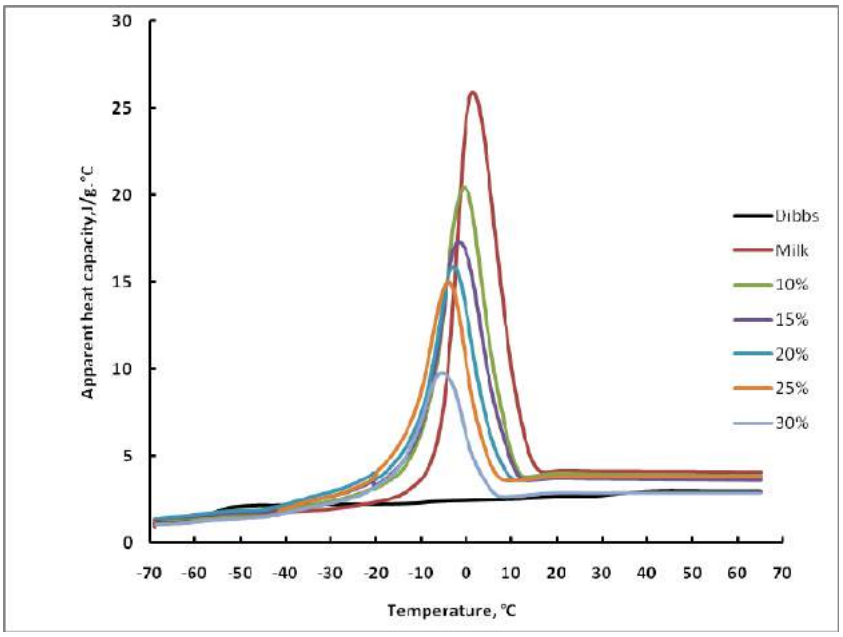
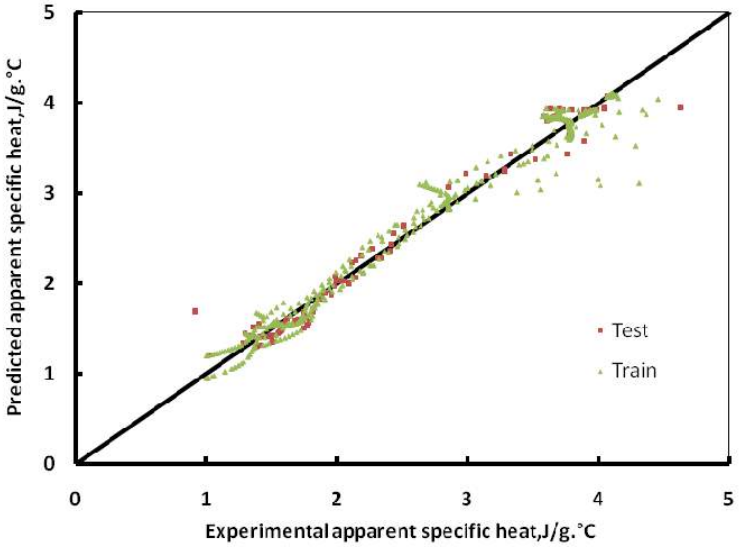


Fig. 3. Apparent specific heat values vs. temperature for the dibbs, milk, and milk-dibbs drinks.

(a)



(b)

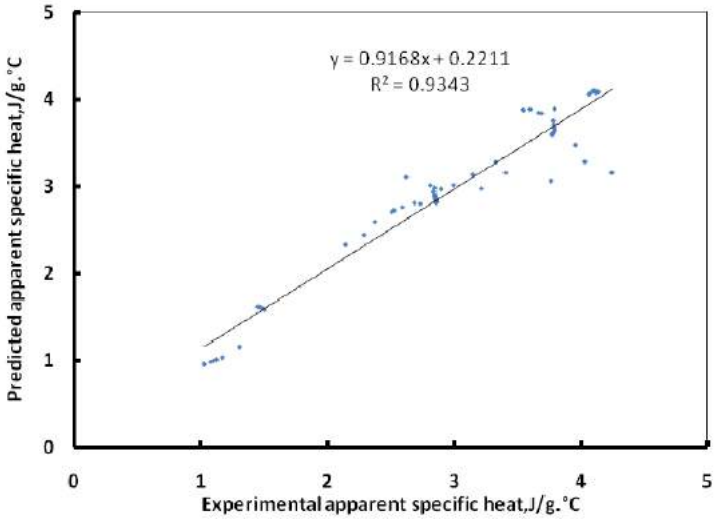


Fig. 4. (a) Experimental vs. predicted apparent specific heat for test and train data sets; (b) experimental vs. predicted apparent specific heat for verification data sets.

Estimation of Some Physico-Chemical Characteristics, Mineral Elements, Sugar Fractions and Amino Acids of Some Aswan Dry-Date Cultivars Produced through Traditional Method (Offshoots) and Tissue Culture Technique

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Keywords: *Phoenix dactylifera* L., traditional method (offshoots), tissue culture technique, 'Gondaila', 'Bartamuda', 'Sakkoti', physico-chemical composition, mineral elements, sugar fractions, amino acid contents

Abstract

This study was carried out to hold a comparison between dates obtained from traditional culture palms as known over the centuries (offshoots), and fruits of tissue culture palms as modern biotechnology which may provide unprecedented opportunities to improve quality parameters and agricultural productivity. Three dry date cultivars namely 'Gondaila', 'Bartamuda' and 'Sakkoti', grown at Aswan Governorate, Egypt, during the 2008-2009 season, were assessed for some physico-chemical characteristics, mineral elements, sugar fractions and amino acids.

Generally, results indicated that tissue culture dates gave the highest values of fruit weight except 'Sakkoti', pulp/fruit percentage, moisture, reducing sugars, total sugars, potassium, magnesium, cobalt, lead and copper while traditional culture dates were superior in crude fiber, total soluble solids, iron, sodium, manganese and phosphorus.

Moreover, both the sugar fractions and the amino acid contents were assessed by HPLC technique. The results revealed that tissue culture dates had the highest percentages in glucose and fructose. In contrast, traditional culture dates were superiority in sucrose to those of tissue culture. The flesh of the tested date cultivars contains many amino acids. Cysteine, histidine, alanine, glycine and glutamic acid were determined. There were variations between traditional dry date cultivars and tissue culture ones in amino acid contents.

Overall, significant differences (at 5% level) in most measured attributes were found between traditional culture dates and tissue ones as propagation method, genotype and interaction between them.

So, production of tissue culture date cultivars should be extended at Aswan Governorate and other successive production areas due to the short life cycle; after plantation (3 years old) they produce dates with high quality.

INTRODUCTION

Date palm tree (*Phoenix dactylifera* L.) is an arborescent of the family *Palmaceae* (*Arecaceae*), inhabit tropical and sub-tropical habitats and the majority of palms are found in the old world, as claimed by Moore (1973). Date palm was known in ancient Egypt 4000 years ago and this fact can be simply proven from date palm inscriptions appearing on the walls of ancient Egyptian temples (Saker and Moursy, 2003).

The importance of the date palm tree was appreciated by many nations over the centuries. This is due to the economic as well as the nutritional value of its fruit; hence it is one of the oldest cultivated tree crops (Fayadh and Al-Showiman, 1990).

Aswan is one of the important areas in Egypt for producing dry dates (Hussein et al., 1979, 1993; Nour et al., 1986; Youssef and Ramadan, 1987; Ramadan, 1995; El-Ghazali and Hussein, 2003; Gadalla, 2003).

The quality and nutritive value of dates are influenced by their chemical

composition (Vandercook et al., 1979). Dates contain essential nutrients, high sugar content as well as moderate percentages of minerals, protein, lipids and vitamins. Moreover, dates have high calorific, nutritive and medical values (Youssef and Ramadan, 1987; Ramadan, 1990, 1995). For the minerals content, date flesh is a very good source of several minerals and could be an important source of potassium for regular consumers (Nizar et al., 2007).

Badawy et al. (2008) studied the protein content of 'Gondaila', 'Bartamuda' and 'Sakkoti' tissue culture dry-dates grown at Aswan Governorate. They revealed that protein % of the previous dates were significantly different. The highest percentage was found in 'Bartamuda' fruits (3.9 and 4.1%), followed by 'Sakkoti' (3.4 and 3.7%) and 'Gondaila' (2.9 and 3.2%) in the seasons 2006 and 2007, respectively.

Increasing date productivity can be achieved through increasing the productivity of existing trees and/or expanding the palm cultivation area. Both ways constitute a big dilemma. Extensive breeding programs for the selection of superior clones through the traditional methods are a tedious effort due to the long life cycle and strongly heterozygous nature of palm in addition to insufficient and expensive offshoots (planting materials) required for new cultivation. Accordingly, there is a dire need for advanced biotechnology as a new emerging technology to solve the previous problems. Nowadays, tissue culture technique is a modern breeding program for date palms breeding (Shaheen, 1990; Aaouine, 1998; Saker and Moursy, 2003).

This work was carried out to assess some of the quality parameters of Aswan dry dates produced from the traditional method (offshoots) and tissue culture technique for some of their physical characteristics, chemical composition, mineral elements as well as fractions of some sugars and amino acids.

MATERIALS AND METHODS

Materials

Three dry dates, i.e., 'Gondaila', 'Bartamuda' and 'Sakkoti' cultivars, all at the tamr stage, for traditional "offshoots" (date palms: more than 20 years old) and counterparts of tissue cultures (10 years old), grown in Al-Akkab village, Aswan Governorate were randomly collected during October, 2008-2009 season. The fresh date fruit pulp was immediately minced, after some physical characteristics were estimated, and packed in small polyethylene bags. These samples were analyzed for different analysis.

Methods

1. Physical Characteristics. Ten fruits from each cultivar were selected randomly, and each individual fruit, representing three replicates, was subjected to physical measurements. Fruit weight (g), flesh weight (g), pulp % fruit and seed weight (g) were determined. Fruit dimension, i.e., fruit length (cm), fruit diameter (cm) and fruit shape (weight/diameter) were estimated using a micrometer caliper. Also, fruit density (g/cm^3), weight/volume, was estimated.

2. Chemical Composition. Moisture, protein, lipids, ash, crude fiber, pH and acidity were determined according to official methods of analysis (AOAC, 1995).

Reducing and total sugars were determined according to the Lane and Eynone method as mentioned in AOAC (1995). Non-reducing sugars were calculated by difference.

Total solids (TS), total soluble solids (TSS) and total insoluble solids (TIS) were determined according to official methods of analysis (AOAC, 1995).

Mineral elements were determined after dry-ashing according to Jackson (1973). Chlorine was determined by titration used silver nitrate. Potassium, sodium, magnesium, calcium, phosphorus, iron, manganese, copper, zinc, cadmium, lead, cobalt and nickel were determined using Unicam SP 1900 Atomic Absorption Spectrophotometer according to Slavin et al. (1975). All chemical parameters were carried out in triplicate.

3. Fractionation of Free Sugars. Several sugars were determined using an HPLC system (HP1050) with a UV detector at 210 nm. The separation was accomplished with a NH_2 (Amino) (5 µm, 4×250 mm) column. The mobile phase consists of (acetonitril/water 76/24 v/v) with 0.1 ml acetic acid. The flow rate was 2 ml/min, while the injection volume was about 10 µl according to the method of Christian (1990).

4. Fractionation of Free Amino Acids. Free amino acids were extracted and determined using an HPLC system (HP 1050) with a UV detector at 254 nm. The separation was accomplished with a ODS, C I 8 (5 µm, 4×250 mm) column. The mobile phase consists of 32% (acetonitril/tetrahydrofuran, 90/10 v/v); and 64% (tetrahydrofuran/water, 5/95 v/v) with 0.3 ml acetic acid and pH was adjusted 5.15 with 1 M NaOH. The flow rate was 1.5 ml/min. The temperature of the column was 60°C, while the injection volume was 10 µl according to the method of Christian (1990).

Data were statistically analyzed using the analysis of variance; means were then compared using the Least Significant Difference (LSD) test at 5% level according to Gomez and Gomez (1984). All estimated parameters were calculated for dry weight basis.

RESULTS AND DISCUSSION

Physical Characteristics

Data on physical characteristics of 'Gondaila', 'Bartamuda' and 'Sakkoti' of traditional method and tissue culture technique are presented in Tables 1 to 3.

It is clear from Tables 1 to 3 that 'Gondaila' and 'Bartamuda' tissue culture fruits had higher weight than those of traditional fruits, by 0.71 and 1.72 g, respectively. In contrast, 'Sakkoti' of tissue dates recorded the lowest weight by 24% compared to counterparts of traditional dates. There was no significant difference in the fruit weight between traditional and tissue culture dates. The results of a comparison among the three cultivars, regardless of propagation method, indicated significant differences. Also, interaction between propagation method and cultivars showed a significant difference.

Pulp % fruit content of tissue culture dates was higher than counterparts those of traditional cultivars. The data indicated that all treatments produced significant values for pulp % fruit.

Fruit lengths of 'Gondaila' and 'Bartamuda' dates are quite similar, higher than 'Sakkoti' by 1.275 and 1.105 cm, respectively. These results are in a good agreement with those reported by Nour et al. (1986) in both 'Bartamuda' and 'Sakkoti'. There was no significant difference between 'Gondaila' and 'Bartamuda' while 'Sakkoti' was significantly lower than both of them. The comparison between traditional and tissue culture dates indicated no significant difference.

The general results showed some differences among the three treatments; propagation method, cultivars and interaction between them; regarding the different studied characters. However, others were not.

Chemical Composition

All possible chemical compositions among treatment means are shown in Tables 4 to 6.

From Tables 4 to 6, it is obvious that moisture content of tissue culture dates was higher than those of traditional cultivars. Total solid percentage followed the opposite trend of moisture percentage for the studied cultivars. Tissue culture dates were significantly higher than traditional ones in moisture content.

It was observed that total soluble solid was higher in 'Gondaila' than in 'Bartamuda' and 'Sakkoti' while 'Sakkoti' recorded the highest value in total insoluble solid. In general, the total soluble solid and total insoluble solid of traditional cultivars were superior to those of tissue culture cultivars. No significant difference in total insoluble solids between traditional and tissue dates was noticed.

The lipid content of the three date cultivars ranged from 1.19 to 1.79% and from 1.07 to 1.59% in the traditional and tissue culture cultivars, respectively. There was a

significant difference between traditional and tissue culture dates in lipid content. 'Gondaila' was significantly higher than 'Sakkoti' and 'Bartamuda'. Results for traditional cultivars are in agreement with those found by Mansour (1974), Hussein et al. (1993) and Al-Khouli et al. (1998), while they showed a lower trend compared to those reported by El-Ghazali and Hussin (2003). Variation in results may be due to the differences in moisture content. Lowering of crude lipid and protein was found in tissue cultivars compared to those of traditional cultivars which may be due to higher content of moisture in tissue cultivars than those found in traditional cultivars (El-Ghazali and Hussin, 2003).

The crude fibers in the three cultivars recorded from 3.14-4.55 in traditional culture and from 2.83-4.28% in tissue culture cultivars. These results are less than reported by El-Ghazali and Hussin (2003), and more than those reported by Hussein et al. (1993). 'Gondaila' contained a higher amount of crude fibers than 'Sakkoti' and 'Bartamuda'. Many researches recorded that those crude fiber contents of the different date cultivars ranged from 1.49-12.00% (Hussein and El-Zeid, 1975; Abdel-Hafiz et al., 1980; Yousif et al., 1982; Khatab et al., 1983; Ramadan, 1995; Hussein et al., 1993; Al-Khouli et al., 1998; El-Ghazali and Hussin, 2003; Gadalla, 2003). 'Gondaila' date cultivar was significant higher than 'Sakkoti' and 'Bartamuda'. There was significant difference between traditional and tissue dates in crude fibers.

Total sugars, reducing and non-reducing sugars were higher in tissue culture than traditional cultivars. The previous results of 'Bartamuda' traditional culture are in agreement with those reported by Hussein et al. (1998) who found that total sugars in 'Bartamuda' were 73.65 and 73.84% in the seasons 1995 and 1996, respectively, while it differs with Al-Khouli et al. (1998), Hussein et al. (1993) and El-Ghazali and Hussin (2003). Observed differences between traditional and tissue culture dates could be attributed to hydrolysis of sucrose and/or respiration (especially at high temperature as in Aswan region, more than 43°C), or combination of both effects, which also can vary among different cultivars (Wills et al., 1998). In a study on chemical composition of 'Bartamuda' and 'Sakkoti' tissue date cultivars, Gadalla (2003) found that reducing sugars were 24.69, 25.42 and 21.47, 21.87% in the seasons 2000 (3 years old) and 2001 (4 years old), respectively. Overall, tissue culture dates were significantly higher than traditional ones.

The three cultivars under investigation contained high ash percentages, it ranged from 2.04 to 2.77 and from 2.66 to 2.90% in traditional and tissue culture cultivars, respectively. Based on the values of the two date sources, the tested cultivars can be arranged in descending order as follows: 'Sakkoti' (2.77 and 2.90%), 'Bartamuda' (2.14 and 2.84%) and 'Gondaila' (2.04 and 2.66%) on the traditional and tissue culture cultivars; respectively. These results for traditional dates are in agreement with those mentioned by Salem and Hegazi (1971), Abdel-Hafiz et al. (1980), Khatab et al. (1983), Ramadan (1995) and El-Ghazali and Hussin (2003) while Hussein and El-Zeid (1975), Hussein et al. (1993) and Al-Khouli et al. (1998) recorded a slight increase in ash contents than those reported in this research, whereas they were 3.30-3.98, 3.05-3.75 and 3.85-4.15% in 'Gondaila', 'Bartamuda' and 'Sakkoti' traditional dates, respectively. In ash statistical analysis, tissue culture dates were significantly higher than traditional ones. Regardless of propagation method, there were significant differences among studied date cultivars. Also, interaction between propagation method and cultivars showed significant differences.

pH values ranged from 6.36 to 6.61, nearly neutralized, and acidity from 0.315 to 0.354. In all date cultivars, while pH values decreased, the acidity contents increased. It is obvious that differences between pH or acidity values in all date cultivars were small and in a safe region, neutral, which reflects a good quality. The results are in accordance with those recorded by Hussein and El-Zeid (1975), Yousif et al. (1982), Hussein et al. (1993), Ramadan (1995), Al-Khouli et al. (1998) and El-Ghazali and Hussin (2003) for traditional date cultivars. Acidity of tissue date cultivars was in the sequence: 'Gondaila' followed by 'Sakkoti' and 'Bartamuda'. These results are in a good agreement with those reported

by Badawy et al. (2008). No significant differences in all treatments for acidity were noticed.

Mineral Elements

Data in Tables 7 to 9 indicate that ash of the tested cultivars was rich in potassium, chlorine, calcium, phosphorus, magnesium and sodium as macro elements while, the concentration of micro elements as iron, copper, manganese, zinc, nickel, cobalt and lead came in the second rank.

It is evident from data in Tables 7 to 9 that potassium was the predominant element present in all date cultivars. 'Sakkoti' date had a higher amount of potassium than 'Gondaila' and 'Bartamuda'. Tissue culture cultivars had a higher amount of potassium than traditional ones. According to Abdessalem et al. (2008), the mineral analysis showed that the studied date cultivars were relatively rich in potassium (283 to 733 mg/100 g) and presented a weak content in sodium (0.6 to 0.9 mg/100 g).

The second high content was chlorine which was found in a higher amount in 'Gondaila' than in 'Sakkoti' or 'Bartamuda'. These results are in agreement with those reported by Yousif et al. (1982) and El-Ghazali and Hussin (2003) for traditional dates. In overall, the chlorine content was higher in traditional date cultivars than in tissue culture ones.

In general, tissue culture date cultivars were superior in calcium and magnesium to traditional culture. In contrast, phosphorus and sodium were higher in traditional date cultivars than in tissue culture ones.

Iron and manganese contents were recorded in a great amount in traditional cultivars. 'Sakkoti' contained a higher amount of them than the other two cultivars in traditional and tissue culture cultivars.

Copper and zinc for traditional and tissue culture cultivars ranged from 0.80-0.85 and 0.27-0.31 mg/100 g and 0.82-0.93 and 0.29-0.37 mg/100 g, respectively. These results are in agreement with those reported by Yousif et al. (1982), Ramadan (1995), Azab (1995) and El-Ghazali and Hussin (2003).

Tissue culture date cultivars contained cadmium, lead, cobalt and nickel in a relatively higher amount than traditional date cultivars; it may be due to the use of chemical pesticides to control insects (Barton et al., 1987; Kramer and Muthukrishnan, 1997; Morton et al., 2000; Valencia et al., 2000).

The variations in mineral elements fluctuation between traditional and tissue culture dates were observed, it may be due to distinct physiological activity for distribution of mineral elements between the pulp and the pit as suggested by Bogner et al. (1990).

An overall look, statistical analysis of mineral elements in all treatments showed significant differences except cadmium and nickel. There were no significant differences in copper, zink and lead for cultivars regardless of propagation method.

Because of genetic differences and variable growth conditions, dates show and their chemical composition, perhaps more than other fruits, wide variations in their final appearance and quality parameters were expected (Ismail et al., 2008).

Sugar Fractions

The sugar fractions were identified into sucrose, glucose, fructose and total polysaccharides hydrolysate (Tables 10 to 12). Data indicated that sucrose fractions recorded a major amount among the total sugars (sucrose, glucose and fructose) in all date cultivars. Glucose/fructose ratios were more than 1 for 'Gondaila', 'Bartamuda' and 'Sakkoti' of traditional and tissue date cultivars. The higher sucrose was found in 'Sakkoti' compared to 'Gondaila' and 'Bartamuda' with significant difference regardless of propagation method. Traditional date cultivars had a high sucrose amount by 1.82% and recorded significantly higher than tissue ones. In opposite, statistically, glucose and fructose were the lowest. It is clear that, reducing and non-reducing sugars determined by HPLC were lower than those determined by fehling volumetric method and a clearget

method with acid inversion, respectively, as shown in Tables 4 and 10. These variations may be due to the presence of colloids and other suspended matters, divalent ions and color matters affect reducing and non-reducing sugar measurements (Saska and Lancrenon, 1994).

Free Amino Acid Fractions

Results of free amino acid determinations (Tables 13 to 15) revealed that fruits of studied date cultivars contain five amino acids, which are determined, and those are cysteine, histidine, alanine, glycine and glutamic acid. The contents of cysteine and histidine were higher in 'Gondaila' and 'Sakkoti' tissue culture dates than counterparts of traditional dates. Also, tissue culture dates were the highest in alanine and glycine. In opposite, 'Bartamuda' and 'Sakkoti' of traditional dates were superior in glutamic acid to tissue ones.

Statistically, the interaction between studied date cultivars and propagation methods recorded significant differences. For propagation method, tissue culture dates were the highest in cysteine, alanine and glycine, while glutamic acid was the highest in traditional dates. There was no significant difference in histidine among them. Regardless of propagation method, 'Sakkoti' was superior in all the determined amino acids, followed by 'Bartamuda' and 'Gondaila' for alanine, glycine and glutamic acid. Cysteine of 'Gondaila' was higher significantly different from 'Bartamuda', while no difference was found between them in histidine.

CONCLUSION

In conclusion, these results cleared the importance of the plant tissue culture technique for propagation of dry-date cultivars, the short life cycle after plantation to produce dates and their quality, grown under Aswan environmental conditions and other successive areas of production.

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Tables

Table 1. Mean values of some physical characteristics of different dry date palm cultivars propagated by tissue culture and traditional method (offshoots).

Physical characteristics	Propagation method×genotype						$LSD_{0.05}$
	Traditional method (offshoots)			Tissue culture technique			
	Gondaila	Bartamuda	Sakkoti	Gondaila	Bartamuda	Sakkoti	
Fruit weight (g)	10.13 c	11.00 b	8.56 d	10.84 b	12.72 a	6.51 e	0.5113
Pulp % fruit	89.34 c	90.46 b	87.61 d	89.87bc	94.18 a	88.15 d	0.7345
Flesh weight (g)	9.05 c	9.95 b	7.50 d	9.74 b	11.98 a	5.73 e	0.6585
Seed weight (g)	1.08 a	1.03ab	1.06 a	1.10 a	0.74 c	0.77bc	0.2636
Fruit length (cm)	5.70 a	5.47 a	4.60 b	5.75 a	5.62 a	4.30 b	0.5753
Fruit diameter (cm)	2.19 a	1.84 b	1.90 b	2.32 a	1.98 b	1.75 b	0.3653
Fruit dimension (ratio)	2.60 b	2.97 a	2.42 b	2.48 b	2.84ab	2.46 b	0.2785
Fruit shape (g/cm)	5.63 a	5.98 a	4.51 b	4.67 b	6.42 a	3.72 b	1.2095
Fruit volume (cm^3)	10.00 b	10.60 b	8.11 c	10.43 b	11.95 a	5.83 d	0.6999
Fruit density (g/cm^3)	1.013 b	1.038 b	1.055 b	1.039 b	1.011 b	1.117 a	0.0655

Table 2. Effect of propagation method on some physical characteristics of fresh Aswan dry-dates.

Physical characteristics	Propagation method		$LSD_{0.05}$
	Traditional method (offshoots)	Tissue culture technique	
Fruit weight (g)	9.897	10.020	N.S.
Pulp % fruit	89.137 b	90.733 a	0.4241
Flesh weight (g)	8.883	9.150	N.S.
Seed weight (g)	1.058 a	0.870 b	0.1522
Fruit length (cm)	5.257	5.223	N.S.
Fruit diameter (cm)	1.977	2.017	N.S.
Fruit dimension (ratio)	2.66	2.59	N.S.
Fruit shape (g/cm)	5.37 a	4.94 b	0.2933
Fruit volume (cm^3)	9.570	9.404	N.S.
Fruit density (g/cm^3)	1.035	1.056	N.S.

Table 3. Effect of genotype on some physical characteristics of fresh Aswan dry-dates (regardless of propagation method).

Physical characteristics	Genotype			$LSD_{0.05}$
	Gondaila	Bartamuda	Sakkoti	
Fruit weight (g)	10.485 b	11.860 a	7.530 c	0.3616
Pulp % fruit	89.605 b	92.320 a	87.880 c	0.5194
Flesh weight (g)	9.395 b	10.965 a	6.615 c	0.4656
Seed weight (g)	1.090 a	0.887 b	0.915 ab	0.1864
Fruit length (cm)	5.725 a	5.545 a	4.450 b	0.4068
Fruit diameter (cm)	2.255 a	1.910 ab	1.825 b	0.4068
Fruit dimension (ratio)	2.54 b	2.91 a	2.44 b	0.3012
Fruit shape (g/cm)	5.15 b	6.20 a	4.12 c	0.9356
Fruit volume (cm^3)	10.215 b	11.275 a	6.972 c	0.4949
Fruit density (g/cm^3)	1.026 b	1.025 b	1.086 a	0.0476

Table 4. Mean values of chemical composition (%) of different dry date palm cultivars propagated by tissue culture and traditional method (offshoots).

Chemical composition	Propagation method×genotype						$LSD_{0.05}$
	Traditional method (offshoots)			Tissue culture technique			
	Gondaila	Bartamuda	Sakkoti	Gondaila	Bartamuda	Sakkoti	
Moisture	13.61de	17.44 b	13.20 e	14.20 d	19.12 a	16.32 c	0.6559
Total solid	86.39 a	82.56 d	86.80 a	85.80 b	80.88 e	83.68 c	0.4381
Total soluble solid	79.10 a	78.20 c	78.10 c	78.50 b	76.10 d	75.90 d	0.2093
Total insoluble solid	7.29 c	4.36 d	8.70 a	7.30 c	4.78 d	7.78 b	0.4343
Lipid	1.79 a	1.19 c	1.57 b	1.59 b	1.07 c	1.20 c	0.1819
Crude fiber	4.55 a	3.14 d	3.93 b	4.28 ab	2.83 d	3.56 c	0.3639
Reducing sugar	20.5 d	23.0 b	15.5 e	22.0 c	24.0 a	21.5 c	0.6783
Non-reducing sugar	55.1 b	50.8 d	56.5 a	56.9 a	53.4 c	54.6 b	0.6223
Total sugar	75.6 c	73.8 d	72.0 e	78.9 a	77.4 b	76.1 c	0.8034
Nitrogen	0.3504bc	0.4240a	0.4384a	0.3440c	0.3872abc	0.4016ab	0.0570
Protein	2.19 e	2.74 b	2.65 a	2.15 e	2.51 d	2.42 c	0.0575
Ash	2.04 f	2.14 e	2.77 c	2.66 d	2.84 b	2.90 a	0.0575
pH	6.36 d	6.57 c	6.45 ab	6.58 ab	6.61 b	6.53 a	0.0573
Acidity*	0.354	0.333	0.341	0.341	0.315	0.322	N.S.

* as citric acid.

Table 5. Effect of propagation method on chemical composition (%) of fresh Aswan dry-dates.

Chemical composition	Propagation method		$LSD_{0.05}$
	Traditional method (offshoots)	Tissue culture technique	
Moisture	14.75 b	16.55 a	0.3787
Total solid	85.25 a	83.45 b	0.2530
Total soluble solid	78.46 a	76.83 b	0.1151
Total insoluble solid	6.78	6.62	N.S.
Lipid	1.52 a	1.29 b	0.1050
Crude fiber	3.873 a	3.556 b	0.2101
Reducing sugar	19.67 b	22.50 a	0.3916
Non-reducing sugar	54.133 b	54.956 a	0.3493
Total sugar	73.80 b	77.47 a	0.4638
Nitrogen	0.4040	0.3780	N.S.
Protein	2.527 a	2.360 b	0.0332
Ash	2.317 b	2.800 a	0.1322
pH	6.460 b	6.573 a	0.0333
Acidity*	0.343	0.326	N.S.

* as citric acid.

Table 6. Effect of genotype on chemical composition (%) of fresh Aswan dry-dates (regardless of propagation method).

Chemical composition	Genotype			$LSD_{0.05}$
	Gondaila	Bartamuda	Sakkoti	
Moisture	13.9000 c	18.2800 a	14.760 b	0.4638
Total solid	86.1000 a	81.8200 c	85.240 b	0.3098
Total soluble solid	78.8000 a	77.1500 b	77.000 c	0.1409
Total insoluble solid	7.3000 b	4.5700 c	8.240 a	0.3071
Lipid	1.6900 a	1.1300 c	1.385 b	0.1286
Crude fiber	4.4150 a	2.9850 c	3.745 b	0.2573
Reducing sugar	21.2500 b	23.5000 a	18.500 c	0.4796
Non-reducing sugar	56.0000 a	52.0800 c	55.550 b	0.4400
Total sugar	77.2500 a	75.6000 b	74.050 c	0.5681
Nitrogen	0.3470 b	0.4060 a	0.420 a	0.0407
Protein	2.1700 c	2.6250 a	2.535 b	0.0408
Ash	2.3500 c	2.4900 b	2.835 a	0.0468
pH	6.470 b	6.4600 b	6.590 a	0.0467
Acidity*	0.3470	0.3240	0.331	N.S.

* as citric acid.

Table 7. Mean values of mineral contents (mg/100 g) of different dry date palm cultivars propagated by tissue culture and traditional method (offshoots).

Mineral elements	Propagation method×genotype						$LSD_{0.05}$
	Traditional method (offshoots)			Tissue culture technique			
	Gondaila	Bartamuda	Sakkoti	Gondaila	Bartamuda	Sakkoti	
Potassium	651.390 d	558.700 f	716.120 c	753.300 b	576.060 e	803.110 a	17.2800
Chlorine	212.010 a	193.390 bc	201.320 b	196.470 bc	170.360 d	188.900 c	10.4400
Sodium	4.110 b	5.330 a	3.340 c	3.030 c	4.790 a	3.110 c	0.6254
Magnesium	25.610 c	28.610 c	35.160 ab	33.760 b	37.500 ab	39.950 a	5.0370
Calcium	116.800 f	160.100 c	136.350 e	179.400 b	209.500 a	144.100 d	4.4560
Phosphorus	106.690 d	122.570 b	138.840 a	100.620 e	114.820 c	123.010 b	4.9000
Iron	4.880 a	3.620 b	5.160 a	2.360 c	2.380 c	2.560 c	0.4343
Manganese	0.380 b	0.280 c	0.440 a	0.220 d	0.160 e	0.320 c	0.0575
Copper	0.850 b	0.800 c	0.800 c	0.930 a	0.820 c	0.900 ab	0.0575
Zink	0.270 b	0.280 b	0.310 b	0.370 a	0.290 b	0.370 a	0.0575
Cadmium	0.012	0.012	0.012	0.013	0.013	0.012	N.S.
Lead	0.029 b	0.026 b	0.026 b	0.081 ab	0.089 ab	0.092 a	0.0575
Cobalt	0.410 b	0.330 c	0.310 c	0.520 a	0.430 b	0.510 a	0.0575
Nickel	0.150	0.140	0.140	0.180	0.180	0.220	N.S.

Table 8. Effect of propagation method on mineral contents (mg/100 g) of fresh Aswan dry-dates.

Mineral elements	Propagation method		$LSD_{0.05}$
	Traditional method (offshoots)	Tissue culture technique	
Potassium	642.100 b	710.800 a	9.9790
Chlorine	202.240 a	185.240 b	6.0250
Sodium	4.260 a	3.640 b	0.3188
Magnesium	29.790 b	37.070 a	0.0407
Calcium	137.800 b	177.700 a	2.5730
Phosphorus	122.700 a	112.700 b	2.8290
Iron	4.553 a	2.433 b	0.3071
Manganese	0.367 a	0.233 b	0.0407
Copper	0.817 b	0.883 a	0.0407
Zink	0.287 b	0.343 a	0.0406
Cadmium	0.012	0.013	N.S.
Lead	0.027 b	0.087 a	0.0407
Cobalt	0.360 b	0.487 a	0.0708
Nickel	0.143	0.193	N.S.

Table 9. Effect of genotype on mineral contents (mg/100 g) of fresh Aswan dry-dates (regardless of propagation method).

Mineral elements	Genotype			$LSD_{0.05}$
	Gondaila	Bartamuda	Sakkoti	
Potassium	702.340 b	567.400 c	759.600 a	12.2200
Chlorine	204.240 a	181.880 c	195.110 b	7.3790
Sodium	3.570 b	5.060 a	3.220 b	0.4130
Magnesium	29.680 b	33.060 b	37.560 a	3.5620
Calcium	148.100 b	184.800 a	140.225 c	3.1510
Phosphorus	103.700 c	118.700 b	130.700 a	3.4650
Iron	3.620 a	3.000 b	3.860 a	0.2508
Manganese	0.300 b	0.220 c	0.380 a	0.0407
Copper	0.890	0.810	0.850	N.S.
Zink	0.320	0.285	0.340	N.S.
Cadmium	0.013	0.013	0.012	N.S.
Lead	0.055	0.058	0.059	N.S.
Cobalt	0.465 a	0.380 b	0.410 b	0.0407
Nickel	0.165	0.160	0.180	N.S.

Table 10. Mean values of sugar content fractions (%) of different dry date palm cultivars propagated by tissue culture and traditional method (offshoots).

Sugar fractions	Propagation method×genotype						$LSD_{0.05}$
	Traditional method (offshoots)			Tissue culture technique			
	Gondaila	Bartamuda	Sakkoti	Gondaila	Bartamuda	Sakkoti	
Sucrose	51.08 b	48.73 d	53.95 a	50.55 c	47.01 e	50.73bc	0.3986
Glucose	11.60 e	13.08 d	9.52 f	14.30 b	16.17 a	13.69 c	0.4381
Fructose	7.80 a	7.93 a	5.21 c	7.20 ab	7.17 ab	6.68 b	0.8401
Total sugars	70.48 c	69.74 d	68.68 e	72.05 a	70.35 c	71.10 b	0.1386
Total polysaccharides	21.40 a	19.82 c	20.52 b	20.30 b	18.05 e	18.73 d	0.3151

Table 11. Effect of propagation method on sugar content fractions (%) of fresh Aswan dry-dates.

Sugar fractions	Propagation method		$LSD_{0.05}$
	Traditional method (offshoots)	Tissue culture technique	
Sucrose	51.25 a	49.43 b	0.2301
Glucose	11.40 b	14.72 a	0.2530
Fructose	6.98	7.02	N.S.
Total sugars	69.63 b	71.17 a	0.0743
Total polysaccharides	20.58 a	19.03 b	0.1819

Table 12. Effect of genotype on sugar content fractions (%) of fresh Aswan dry-dates (regardless of propagation method).

Sugar fractions	Genotype			$LSD_{0.05}$
	Gondaila	Bartamuda	Sakkoti	
Sucrose	51.27 b	47.87 c	51.79 a	0.2818
Glucose	12.95 b	14.63 a	11.61 c	0.3098
Fructose	7.50 a	7.55 a	5.95 b	0.7356
Total sugars	71.72 a	70.05 b	69.44 c	0.0910
Total polysaccharides	20.85 a	18.94 c	19.63 b	0.2228

Table 13. Mean values of amino acid fractions (mg/100 g) of different dry date palm cultivars propagated by tissue culture and traditional method (offshoots).

Amino acid fractions	Propagation method×genotype						$LSD_{0.05}$
	Traditional method (offshoots)			Tissue culture technique			
	Gondaila	Bartamuda	Sakkoti	Gondaila	Bartamuda	Sakkoti	
Cysteine	34.24 d	28.80 de	72.00 c	109.83 a	23.12 e	97.66 b	6.025
Histidine	4.92 e	25.41c	49.80 b	13.73 d	0.78 e	67.66 a	7.157
Alanine	65.30 e	73.62 d	93.40 c	100.20 c	135.17 b	144.15 a	7.247
Glycine	16.80 e	25.13 d	26.64 cd	29.19 c	35.72 b	39.43 a	3.187
Glutamic acid	5.81 e	21.33 c	39.70 a	12.83 d	8.19 e	33.47 b	3.844

Table 14. Effect of propagation method on amino acid fractions (mg/100 g) of fresh Aswan dry-dates.

Amino acid fractions	Propagation method		$LSD_{0.05}$
	Traditional method (offshoots)	Tissue culture technique	
Cysteine	45.01 b	76.87 a	3.478
Histidine	26.71	27.39	N.S.
Alanine	77.44 b	126.51 a	4.184
Glycine	22.86 b	34.78 a	3.069
Glutamic acid	22.28 a	18.16 b	2.219

Table 15. Effect of genotype on amino acid fractions (mg/100 g) of fresh Aswan dry-dates (regardless of propagation method).

Amino acid fractions	Genotype			$LSD_{0.05}$
	Gondaila	Bartamuda	Sakkoti	
Cysteine	72.040 b	25.960 c	84.830 a	4.260
Histidine	9.325 b	13.095 b	58.730 a	5.061
Alanine	82.750 c	104.400 b	118.780 a	5.124
Glycine	22.990 c	30.430 b	33.030 a	2.254
Glutamic acid	9.320 c	14.760 b	36.585 a	2.718

Microbiological Analysis of Date Palm Fruit Sold in Abu Dhabi Emirate

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Keywords: HACCP, date palm, osmophilic yeast, mould, fruits, *E. coli* MPN

Abstract

ADFCA act to monitor the implementation of hygiene during the manufacturing process following the HACCP system and the application of food safety requirements.

This is to ascertain that the date fruit is of high quality and free of microbiological contamination and to ensure healthy products for human consumption through testing at approved laboratories in ADFCA.

Samples of date palm fruits (*Phoenix dactylifera* L.) of different date palm cultivars including bulk, loss, vacuum, paste and date with chocolates were collected from local markets, retailed products and processed by-products from factories in Abu Dhabi emirate brought to the laboratories, Microbiology section in Abu Dhabi Food Control Authority (ADFCA), Al Ain branch.

The microbiological quality of date fruit was studied following standard operation procedure (1), LAB-DPM-MICRO-09 for enumeration of yeast and mould in food, using wort agar for isolation of osmophilic yeast and potato dextrose agar for mould identified by macroscopic appearance on selective culture media and microscopic morphology, other method of LAB-DPM-MICRO-03 for detection of *Salmonella* spp. in food by pre-enrichment culture and sub cultured on selective media and LAB-DPM-MICRO-07 for enumeration of coliform bacilli and *E. coli* in food by MPN technique using appropriate enrichment broth for *E. coli* and coliform cultivation by sub culturing on selective media.

Through periodic inspections, ADFCA have been able to reduce the rate of microbiological contamination by osmophilic yeast and molds from 29.7% in 2006 to 16.6% in 2009 by work of investigation in terms for correct application of requirements' specifications from import institutions and factories also application of standard measurement rules by damage of cargo in case of unfit microbiological contamination products. A total number of (245) samples , 53 (21.6%) samples were not compliant with UAE regulation results due to total count of osmophelic yeast and mould exceeding the maximum limit according to microbiological criteria in the UAE standard No.: 1016/2002, (2), ranging from 29.7% (22/74), 19.5% (9/46), 21.7% (5/23) and 16.6% (17/102) in 2006, 2007, 2008 and 2009 respectively, while *E. coli* and *Salmonella* were not detected in this study.

INTRODUCTION

Scientific name: *Phoenix dactylifera* L., the date palm, has been a stable food for the population of the Middle East and North Africa for thousands of years. Date fruit is a drupe with a single seed or pit. Dates are a high-energy food containing 3000 calories/kg and offer a good source of high nutritive value and many people today consume dates as a ready to eat snack. However, it still retains its symbolic significance, and is widely used to break the fast during the month of Ramadan (UAE University website).

Fruit growth follows a sigmoid curve, and it is usually divided into five stages of development known by their Arabic terms: "hababouk," "kimri," "khalal," "rutab," and "tamer". Most of the dates are harvested in the "tamer" stage, when the fruit has about 60

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to 80% sugar content depending on location and cultivars. At this stage, fruits can be harvested soft, semi-dry or dry depending on destination and use.

The most common pathological deterioration of dates includes fermentation by yeast (most important) and molding by fungi. Therefore a good storage technique is necessary to keep the date fruit in its best quality. Refrigerators have been found to prolong the shelf life of the fruits since the fungi and enzymes which act on the dates require an average temperature of 25-30°C, the fruits are kept at much lower temperature to inhibit their activities.

Heating and direct sun drying are ways of dehydrating the fruit to reduce the moisture level to the minimum. Steam-hydrated dates are more resistant to attack by microorganisms than natural or non hydrated dates because of the partial sterilization of steam dehydrated fruit. These fungi may cause significant losses before or just after harvest during rainy or high RH periods and can attack fruits at the "khalal" or "rutab" stages. However, most of these fungi will not grow on dried dates (Kader and Hussein, 2009).

The objectives of this study were to investigate microbiological profiles so as to know the hygienic conditions of the date fruits.

MATERIAL AND METHODS

Sampling

Samples of date palm cultivars' fruits including bulk, loss, vacuum packaged, date pastes and date chocolates were obtained by ADFCA inspectors from different sources of exhibits in local markets, importer and exporter factories from Al Ain state of UAE from January 2006 to August 2009, brought to the department of laboratories, microbiology section in Abu Dhabi Food Control Authority (ADFCA), Al Ain branch.

These cultivars are 'Khadraoui', 'Khlass', 'Medjool', 'Nabt-Saif', 'Sukkari', 'Umal dahen', 'Maktoumi', 'Rziz', 'Buklat Al dahla', 'Barhee', 'Abu mann', 'Mabroom', 'Farth', 'Maknooze Durah', 'Lulu', 'Chichi', 'Safawi', 'Sultana', 'Nmishi', 'Zamli', 'Sayer', 'Khenezi', 'Jabri', 'Safri', 'Nghal', 'Rashidi', 'Dabbas', 'Berni', 'Nawat Al barkh', 'Dayri', 'Zahidi', 'Khdri' and 'Sakii'.

Analysis

LAB-DPM-MICRO-09 SOP was applied for enumeration of yeast and mould in food by aseptically weighing 20 g diluted and homogenate in 180 ml of appropriate diluents, inoculating using wort agar for isolation of osmophilic yeast (*Zygosaccharomyces*) and potato dextrose agar for mould. Aliquots (1 ml) of decimal dilution extract for inoculation on two plates for both selective media, incubation at 30°C for 72 h, were characterized based on their macroscopic appearance on culture medium, microscopic morphology examination of isolated colonies for yeast and mould (Omogbal et al., 2007).

LAB-DPM-MICRO-03 SOP for detection of *Salmonella* spp. in food, cultivation in pre-enrichment culture buffer peptone water broth were applied by aseptically weighing 25 g diluted and homogenates in 225 ml of pre-enrichment broth then incubated for 24 h at 37°C, subcultured on two types of secondary enrichment broth by inoculating a liquate 0.1 ml and 1 ml in *Rappaport vasseliadis* and Tetrathionate broth respectively, incubated for 24 h at 37°C, then subcultured on selective media XLD agar and Brilliant green agar. Positive results give characteristic growth colonies on selective agar confirmed by biochemical tests (Abu Dhabi Food Control Authority).

LAB-DPM-MICRO-07 SOP for determining of coliform bacilli and *E. coli* in food by MPN technique. Aliquots (1 ml) of three dilution extracts for inoculation in lauryl tryptose broth incubated for 24-48 h at 37°C subcultured in brilliant green bile broth and Mug trypton water and incubated for 24-48 h at 44.5±0.5°C for cultivation of *E. coli*, subcultured on selective agar MacConkey agar, Eosin methylene blue agar, distinct colonies from selective agar for pure cultures of bacteria isolates characterized identified

on the basis of their cultural morphological and biochemical properties.

RESULTS

From a total number of (245) samples, 53 (21.6%) samples were not compliant with UAE regulation results due to total count of osmophelic yeast and mould exceeding the maximum limit according to microbiological criteria in the UAE standard No.: 1016/2002. The rate of unfit fruit dates ranged from 29.7% (22/74), 19.5% (9/46), 21.7% (5/23) and 16.6% (17/102) in 2006, 2007, 2008, 2009 respectively. *E. coli* and *Salmonella* were not detected in this study as shown in Figures 1 and 2.

DISCUSSION

Dates (*Phoenix dactylifera* L.) is an important product in many Arabic countries. Date palm is becoming an important commercial crop in the Arabian Peninsula (AP). In these producing countries significant increases in yield are being achieved through adoption of advanced biotechnology approaches. There has been renewed interest in the date as a component of new food formulations and preparations. Process industries manufacture a variety of date products such as date-paste, date-syrup, date dip, date honey, date jam.

Of microbiological characteristics, yeasts, moulds, coliform, *E. coli* and *Salmonella* are the most important factors for determining the overall quality and consumer acceptability of fresh fruits and vegetables. Microbiological properties are an essential analytical measure, providing fundamental insight in the conservation of food (Mrabet et al., 2008).

Through periodic inspections, ADFCA have been able to reduce the rate of microbiological contamination of dates by osmophilic yeast and molds from 29.7% in 2006 to 16.6% in 2009 by investigation in terms of correct application of requirements' specification from importing institutions and factories and also application of standard measurement rules by damage of cargo in case of unfit microbiological contamination products.

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Figures

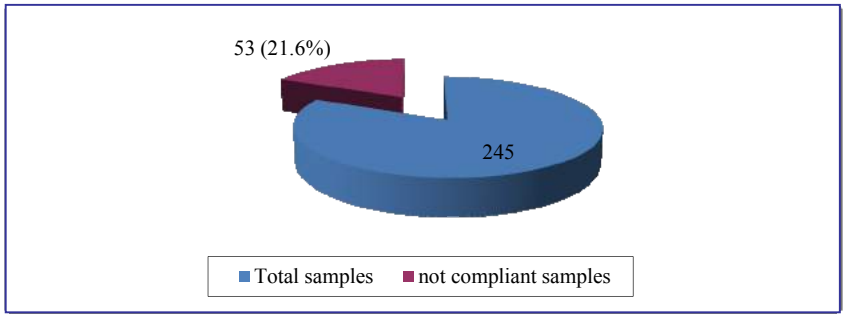

Fig. 1. Percentage of non compliant number from total samples.

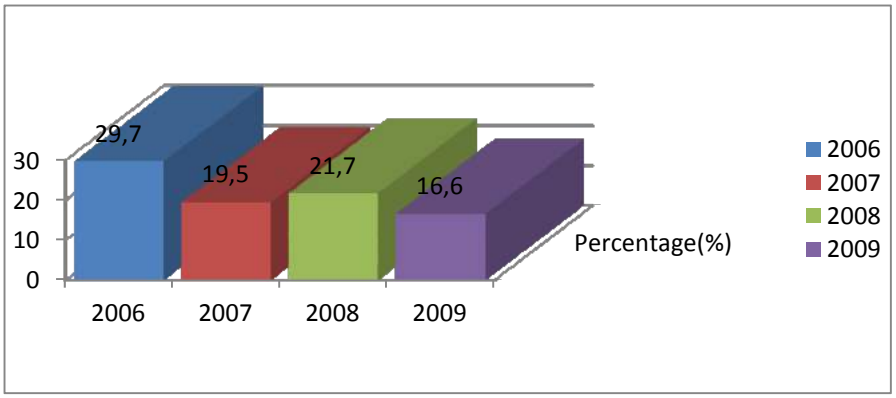

Fig. 2. Percentage of contamination (yeast and molds) of non compliant samples according to the years 2006-2009.

Effect of Bunch Bagging Color on 'Succary' and 'Khalas' Date Palm Cultivars: Fruit Chemical Characteristics

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Keywords: 'Succary', 'Khalas', *Phoenix dactylifera* L., bunch bagging

Abstract

A field study was carried out during two successive seasons (2007 and 2008) at the Agricultural Research and Experiment Station, Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh Saudi Arabia. Two date palm cultivars, 'Succary' and 'Khalas' bunches were subjected to five plastic bagging color treatments: black, white, blue, yellow, and control (unbagged) in order to study the effect of bunch bagging color on harvest periods, yield and fruit quality. Results showed that the bagging treatments improve fruit chemical properties compared to unbagging. Blue bags significantly increased the fruit total soluble solids, reducing, non-reducing sugars of 'Succary' and 'Khalas' cultivars in both seasons.

INTRODUCTION

One of the world's first cultivated fruit trees, the date palm (*Phoenix dactylifera* L.) was domesticated in Mesopotamia. It has long been one of the most important plants of arid desert areas of northern Africa, the Middle East and southern Asia, appropriately called "the tree of life". It has provided food, ornamentals, material for shelter, fiber, and fuel. It has even been used for religious purposes in a harsh environment where relatively few other plants can grow (Zaid, 2002; Hodel and Johnson, 2007). The date palm is the most widely grown fruit in Saudi Arabia. Annual production is estimated at 986,409 tons of more than 400 different date cultivars, produced from 23,458,299 trees (Agriculture Statistical Year Book, 2009). 'Succary', 'Khalas' and 'Barhee' date palm are the most important cultivars in Saudi Arabia. Date palm bunch covers offer several advantages and are commonly used in the date palm culture areas in order to protect fruits from high humidity, rain, bird attacks, blemishes caused by wind-blown dust and also from damage caused by insects. Al-Baker (1972) reported bagging spathes for 30 days after pollination with date fibers, as used in El-Ahsa, Saudi Arabia from old times. Also, Nixon and Carpenter (1978) reported that no damage has been observed from using bags. El-Kassas et al. (1995), El-Salhy (2000) and Rabeh and Kassem (2003) reported that bunches bagging of 'Zaghloul' and 'Samani' showed a beneficial effect concerning fruit set, yield and physical and chemical fruit properties. In addition, such treatment exhibited the highest fruit weight, flesh weight, fruit dimensions, total soluble solid percentage, total sugars percentage and lowest tannins percentage (Moustafa, 2007; Alshariqi et al., 2007).

MATERIALS AND METHODS

The present study was carried out during the 2007 and 2008 growing seasons on ten-year-old 'Succary' and 'Khalas' date palm cultivars grown in the Agricultural Research and Experiment Station, Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. Five palms were selected for each cultivar. The experimental palms were healthy, as they were uniform in growth, vigor, height and fruiting capacity in the preceding years. Only 10 bunches were left on each experimental

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tree. All cultural practices were carried out according to the applied schedule for experimental palms. Pollination was achieved by using pollen grains from the same parent in both seasons. The experiment was arranged in a complete randomized block design and each treatment was replicated ten times. For each treatment, two bunches per tree were used. The treatments were as follows: four bagging colors, bagging by black plastic bag, white, blue, yellow and unbagged as control. All bagging treatments were applied after four weeks from pollination in both seasons. The bags' size was 100 cm L × 85 cm W and perforated with 300 perforations (0.4 cm^2 for each). The total perforation was 1.41% of the total bag area. The bags were removed from bunches when the fruit started coloring. At the tamar stages 50 fruits per bunch were randomly collected and immediately transported to the Fruit Laboratory of the College of Food and Agricultural Sciences for fruit chemical properties: moisture content, total soluble solids %, acidity %, reducing sugars, non-reducing sugars, total sugar according to AOAC (2000).

The collected data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1980). Means were compared by the Least Significant Difference test (LSD) at the 5% probability level for both seasons.

RESULTS AND DISCUSSION

Total Soluble Solids

Data in Tables 1 and 2 indicate that bagging treatments significantly increased the total soluble solids percentage of 'Succary' and 'Khalas' in the 2007 and 2008 seasons. The highest percentage of total soluble solids was found with blue color treatment of 'Succary' and 'Khalas' compared to the control and other treatments in both seasons. 'Succary' dates had the highest values of total soluble solids compared to those in 'Khalas' cultivar in both seasons. The covered bunches had more total soluble solids than the control one, probably because the higher temperature under the cover favored the conversion of starch into sugars. Parmar and Chundawat (1984) and Reddy (1989) also reported similar findings. The reduction in the content of total soluble solids in control fruits might be due to the higher moisture content of these fruits (Tables 1 and 2). The previous results are in accordance with those found by Awad (2007), El-Salhy (2000) and Moustafa (2007). They reported that bagging treatments increased total soluble solids.

Reducing Sugars

Data presented in Tables 1 and 2 indicate that bagging treatments caused a significant increase in reducing sugars compared with the unbagging (control) of 'Succary' and 'Khalas' in 2007 and 2008. Blue bags followed by black bags gave the highest values of reducing sugars for 'Succary' and 'Khalas' cultivars compared to the control and other bagging treatments in both seasons. 'Khalas' dates had higher reducing sugars than the 'Succary' dates in both seasons.

Non-Reducing Sugars

Non-reducing sugars percentages were significantly affected by applying bagging treatments for 'Succary' and 'Khalas' date palm cultivars in both seasons (Tables 1 and 2). Blue bags gave the highest values of non-reducing sugars as compared with the other applied treatments and control in both seasons. 'Succary' had the highest significant value of non-reducing sugar compared to 'Khalas' in both seasons. However, the non-reducing sugars percentages were not significantly affected by applying bagging treatments for 'Khalas' date palm cultivar in both seasons.

Total Sugars

The obtained results (Tables 1 and 2) indicated that the total sugars percentage was significantly affected by bagging treatments in both seasons. Blue bags gave the highest total sugars percentage as compared with the control and other treatments in both seasons. 'Succary' dates had the highest total sugars percentage as non-reducing sugars.

However, 'Khalas' dates had the highest values for reducing sugars from total sugars percentages in both seasons.

Total Acidity

The obtained results (Tables 1 and 2) indicated that the total acidity percentages were not significantly affected by bagging treatments of 'Succary' and 'Khalas' in 2007 and 2008.

Moisture Content

Data presented in Tables 1 and 2 indicate that bagging treatments caused a significant decrease in moisture content of 'Succary' and 'Khalas' in 2007 and 2008. The control (unbagging) had a higher moisture content percentage compared to the other treatments in both seasons. However, the blue bag color gave the lowest moisture content percentage compared to the control and other treatments. These results are in agreement with El-Kassas et al. (1995), El-Salhy (2000), Mahmoud et al. (2003) and Moustafa (2007). They reported that the bagging of bunches improved chemical characteristics.

From this study, it could be recommended that bagging the date palm bunches four weeks after pollination using a blue color cover must be done to improve fruit quality.

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Tables

Table 1. Effect of bagging color on fruit chemical characters of 'Succary' and 'Khalas' date palm cultivars in 2007.

Cultivars	Bagging color	TSS (%)	Reducing sugars (%)	Non-r. sugars (%)	Total sugars (%)	Acidity (%)	Moisture content (%)
	Control	60.29	23.17	31.74	54.91	0.220	19.23
	Black bags	63.32	24.27	32.90	57.17	0.228	15.33
	White bags	60.22	22.89	31.29	54.18	0.225	14.57
	Blue bags	66.00	27.92	33.57	61.49	0.232	15.53
	Yellow bags	61.49	25.40	31.99	57.39	0.225	15.07
$LSD_{0.05}$ for bagging		0.89	0.77	1.21	0.98	ns	0.63
Succary		63.08	12.13	46.29	58.41	0.204	16.64
Khalas		61.99	38.55	18.71	57.26	0.247	15.85
$LSD_{0.05}$ for cultivar		0.56	0.49	0.77	0.62	ns	0.39
	Control	60.23	10.84	44.36	55.20	0.198	17.53
	Black bags	64.43	11.43	46.48	57.91	0.207	16.63
Succary	White bags	62.10	12.23	46.07	58.30	0.205	16.43
	Blue bags	66.73	14.24	48.57	62.81	0.210	15.97
	Yellow bags	62.10	11.88	45.95	57.83	0.202	16.63
	Control	60.35	35.50	19.12	54.62	0.249	20.93
	Black bags	62.20	37.10	19.34	56.44	0.249	14.03
Khalas	White bags	61.33	39.65	18.51	58.16	0.245	14.71
	Blue bags	65.27	41.60	18.57	60.17	0.253	15.09
	Yellow bags	60.87	38.92	18.02	56.94	0.247	14.50
$LSD_{0.05}$ for cultivar×bagging		1.26	1.01	1.71	1.21	ns	0.88

Table 2. Effect of bagging color on fruit chemical characters of 'Succary' and 'Khalas' date palm cultivars in 2008.

Cultivars	Bagging color	TSS (%)	Reducing sugars (%)	Non-r. sugars (%)	Total sugars (%)	Acidity (%)	Moisture Content (%)
	Control	61.45	23.33	30.67	54.00	0.223	19.34
	Black bags	64.00	25.76	32.33	58.09	0.230	15.60
	White bags	63.34	25.95	32.54	58.49	0.227	15.86
	Blue bags	66.40	27.69	33.95	61.64	0.235	15.85
	Yellow bags	62.60	24.77	31.48	56.25	0.227	15.89
$LSD_{0.05}$ for bagging		0.76	0.66	1.37	0.97	ns	0.71
Succary		64.11	12.33	45.76	58.09	0.207	16.63
Khalas		63.00	38.67	18.02	57.29	0.249	16.38
$LSD_{0.05}$ for cultivar		0.48	0.42	0.87	0.61	ns	0.34
	Control	61.64	11.16	42.84	54.00	0.201	18.17
	Black bags	64.87	11.41	46.42	57.83	0.207	16.13
Succary	White bags	63.87	12.54	46.11	58.65	0.208	16.17
	Blue bags	67.72	14.52	49.45	63.97	0.213	16.40
	Yellow bags	62.47	12.01	44.00	56.01	0.205	16.28
	Control	61.25	35.50	18.50	54.00	0.244	20.50
	Black bags	63.13	40.11	18.24	58.35	0.251	15.06
Khalas	White bags	62.80	39.35	18.96	58.31	0.247	15.55
	Blue bags	65.07	40.85	18.45	59.30	0.256	15.29
	Yellow bags	62.73	37.52	18.95	56.47	0.249	15.49
$LSD_{0.05}$ for cultivar×bagging		1.10	0.94	1.94	1.37	ns	0.40

Study on Effects of Bunch Covering on Date Palm Bunch Fading in Iran

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Keywords: bunch covering, date fading, mechanical control, Iran

Abstract

The date palm bunch fading disorder (DPBF) is the most harmful and hazardous problem in date palm plantations in southern provinces of Iran such as Hormozgan, Bushehr, Fars, Khuzestan and Kerman in the last 10 years. This disease reduces up to 85% of yield on the cultivar 'Mozafti' in Hormozgan and almost 33% of yield on 'Kabkab' in Bushehr from 1998 until now. Looking at past research, a principal agent for this phenomenon has not been found but the fungus *Thielaviopsis paradoxa* induces severe disease as an animate agent in some areas (e.g., Hormozgan). Many researchers believe that environmental hard conditions including hot winds, stress of low humidity and dust haze affect the occurrence and severity of this disease. This factorial experiment was carried out in a complete randomized blocks design (CRBD) with five treatments of kinds of bunch covers (plastic, mat, hemp and cloth bags) and control without any cover and two treatments of time of covering (at kimri and khalal stages) in eight replications, on eight 12-year-old trees of the susceptible cultivar 'Mordaseng' in Roodan region in 2001-2003. The yields of experimental plots were harvested at August and damages were evaluated by counting wilted and dried fruits in each strand, which showed DPBF symptoms. Two years results showed that damage occurred in plastic cover 23.88%, mat cover 31.11%, cloth cover 34.57% and hemp cover 49.31% and in control treatment without cover 58.31%. Two times treatments of covering did not show any significant difference.

INTRODUCTION

Date palm plantation areas in Hormozgan province are estimated about 33,662 and in Iran about 218,000 ha with up to 400 cultivars. 'Mordaseng' and 'Piarum' are dominant in Hormozgan region. Different factors including unfavorable climatic conditions, poor orchard management, high abundance of growing trees in each unit of area and already in 8-9 recent years the DPBF disease are most important agents to reduce quality and quantity of date fruit yields in Iran. The DPBF disease at first was reported in Jiroft region of Kerman province in 1996. Then this disorder damage was reported on 'Kabkab' and 'Shahaby' in Boushehr province in 2000 and after one year the disease (DPBF) was reported on 'Mordaseng' and 'Karite' in Hormozgan province (Avand Faghih, 1999; Karampour, 2002; Mohebbi, 2000). Looking back at recent years research, animate agents especially thermophillic fungi such as *T. paradoxa* and inanimate agents such as hard climatic factors (high temperature, hot winds, stress of very low relative humidity in orchards, poor fertilization and irregular low irrigation) are very effective on occurrence and severity of DPBF (Karampour, 2002; Mohebbi, 2000). The effects of different date bunch covers on damages of birds, climatic factors and harmful insects were evaluated by many researchers in the world. Mohamadpour and Pejman (2001) studied the influence of protecting date bunch covers on quality characters of date fruits and reduction of damages by wasps and *Carpophilus* spp. on commercial date cultivars. They reported that the wire gauge cover reduced fruit defoliation by 4.6% in comparison to the control without cover. Bliss (1938) studied cloth and kraft papers with/without holes and metal circles on prevention of birds, insects and fungal rots on 'Daglat Nour'

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fruits. He reported that combination of whole covers with a metal circle had the most influence compared to other treatments. Gudebec and Hunter (1928) evaluated the effects of cloth covers against rain damages on date bunch. They covered date bunches in late khelal stage with calico bags and finally reported that these covers affected to reduce fungal and bacterial rots, birds and insects damages. Sharples and Hilgeman (1951) studied the influence of cotton cloth and kraft bag covers on date fruit shriveling and blacknose disorders and reported that the short and long kraft and cloth bags had an effective role on damages respectively and they caused an increase of date fruits quality. Eeta (1991) studied the effects of five kinds of bunch covers (hemp, plastic, oil paper, metal and cloth bags) in comparison with control (without cover) to reduce damages of climatic agents and birds from pollination until date fruit ripening stages on quality and quantity characters of date fruit yield. His experimental results showed that metal and cloth bags had preferable effects in comparison to the control treatment respectively. Because of the low cost and simple application, he recommended the cloth covers. Al-Baharany et al. (1994) showed that application of hyaline plastic covers on date bunches before fruit harvest, reduces fruit defoliation and finally increases yield by reducing birds and insects damages. In two separated experiments, the effects of aluminum cover, thick cloth, thin cloth and kraft paper covers were carried out on DPBF in Jiroft region by Karimipour Fard (2001). The results showed that the highest damages occurred in thin cloth and the control treatment (without cover). Moderate damages happened in thick cloth and kraft paper bags and lowest in aluminum covers. Also the same experiment showed that just aluminum cover on bunch peduncle causes a reduction of DPBF and the other covers such as cloth, date palm fibril and oily white color covering on bunch peduncle did not have significant effect on this disorder in comparison to the control. He also reported the effect of climatic factors, particularly very low humidity, high temperature and dry hot western northern winds on occurrence and development of DPBF. Bliss (1938) studied the influence of paper covers with various colors and combinations of paper and cloth covers on reduction of birds, insects and rain damages in different growth stages of 'Daglat Nour' date fruits. The hyaline plastic covers are used for date fruits protection against rain damages and spoilage in Tunisia now. This cover causes an increase in temperature almost 6-8°C and a decrease in humidity about 8% around bunches and finally cause fast ripening of date fruits (Mohammadpour and Pejman, 2001). Application of plastic bags on date bunches at khelal stage in the north of Aghaba date plantations cause an increase in fruit quality on 'Daglat Nour' (Sanadgol, 1991).

MATERIALS AND METHODS

Materials

1. Mat cover bags made by date fibers and leaflets.
2. Hemp cover bags made by cannabis.
3. Plastic cover bags made by nylon matter.
4. Cloth cover bags made by calico+lace matters with a zip on the down side.
5. Control without any cover.

Also the chemical Omite was applied against *Oligonychus afrasiaticus* as a common hazardous pest in all treatments.

Methods

This study was carried out in Roodan region date plantations on 'Mordaseng', which had infected date palms in the past three years. Among the trees that were cultivated in rows, there were eight 10-12-year-old trees selected and nine bunches on each tree recognized as experimental treatments. All of the treatment trees were treated by 1.5/1000 L Omite solution against *O. afrasiaticus* in two times. This factorial experiment was carried out in a randomized completed block design (RCBD) with five treatments of covering kinds and two treatments of covering times (the first at kimri stage in May and

the second at early khelal stage in July 2001). Fruit thinning was done in 1:8 rate (1 bunch/8 green leaves) after first covering stage. All treatments were evaluated after fruit coloring stage (khelal) very carefully. The first symptoms of DBFD were observed on 17th July slowly in first week and developed fast in the 2nd and 3rd weeks. Finally all bunches were harvested on 31st August and damages were evaluated as follows: 15 strands of each bunch were selected randomly and the amount of infected strands was counted. Also the amount of infected fruits per each strand were counted and registered. The disease damage percentages were evaluated, crude data were analyzed statistically by MSTAT-C software and means comparisons were done by Duncan's multiple range test.

RESULTS

Two years combinative analysis and means comparison were done for two factors at two stages of covering separately, percentage of infected strands per each bunch and percentage of infected fruits per each strand. The results obtained are as follows.

Damage Percentage of Infected Strands

The results showed that covering had a significant effect on amount of damage at 1% probability level (Table 1). The lowest amount of damage occurred with plastic bag covers (23.98%) then in mat covers (31.11%) and the highest damage happened in the control (58.31%) then in hemp covers (49.31%). The calico-lace combination covers had a moderate amount of damages (34.57%) among the other cover treatments. The effect of times of covering treatments did not have significant difference on damage of DBFD. Also the reciprocal effects of year×covering time, year×kind of covers and year×covering time×kind of cover are observed in Figures 1-5.

According to these two years experimental data the following results are obtained:

- Effect of year was significant at 1% probability level.
- Effect of replication was significant at 1% probability level.
- Effect of time of covering was not significant.
- Reciprocal effect of year×time of covering was not significant.
- Effect of kinds of covering was not significant at 1% probability level.
- Reciprocal effect of year×kinds of covering was not significant.
- Reciprocal effect of time×kinds of covering was not significant.
- Reciprocal effect of year×time×kinds of covering was not significant.

Damage Percentage of Infected Fruits

The results of experiment in two years showed that bunch covering had an effect on the amount of infected fruits, significantly at 1% probability level (Table 2).

In accordance with these two years experimental data, the following results were obtained:

- Effect of year was significant at 1% probability level.
- Effect of replication was significant at 1% probability level.
- Effect of time of covering did not have significant difference.
- Reciprocal effect of year×time of covering was not significant.
- Effect of kind of cover did not have significant difference.
- Reciprocal effect of year× kind of cover was not significant.
- Reciprocal effect of time kind of cover was not significant.
- Reciprocal effect of year× kind× time of covering was not significant.

CONCLUSION AND RECOMMENDATIONS

The results of this study show that use of bunch covering is effective on date bunch fading disease (DBFD) in Roodan area with warm and semi-dry weather and reduces damages of DBFD. These results correspond with the report of Mohammadpour and Pejman (2001) about the use of bunch covering for reducing of damages of wasps, acari pests and fruit defoliation in Hormozgan province. In this research plastic bags and mat covers reduced damage of DBFD in comparison with the control treatment (without

cover), hemp cover and combination of calico-hence cover respectively. These results also correspond with reports of Eeta (1991), Karimipour Fard (2001), Al-Baharany et al. (1994), Bliss (1938), Gudebec and Hunter (1928) and Sharples and Hilgeman (1951). They all recommended use of bunch covering to reduce damage of unsuitable climatic agents, birds and other pests. The weather statistical data show a clear difference of temperature and relative humidity in May, June, July and August in the first experimental year (2001) in comparison with the second year (2002). These facts describe reciprocal effect of kinds of covers on percentage of damages on infected strands and infected fruits in two years of this research. The results of this research show that application of correct managing methods, especially bunch covering before harvest stage, are very important and useful basic programs in date fruit production. In addition to chemical control, fruit thinning and bunch covering are effective cultural and mechanical measures against insect pests, mites, birds and other harmful general pests in a high qualitative and quantitative date fruit production program. In the old world, especially in Iran and other date palm growing countries, bunch covering with mat covers was used many years, covers which were made by themselves of palm leaves. But date growers have forgotten this simple and cheap method in recent years unfortunately. Because basic materials of mat covers (palm leaves) are very abundant and simply available to date growers, and because these bags are not made by harmful but by natural materials, they can make them by their family cooperatively. The mat covers are very cheap economically, not harmful to the environment, so simple to use and handle, having multiple use objectives and many other positive characteristics. Therefore we recommend these bags as a measure to reduce date bunch fading disease. This method causes very safe fruits with high quality and quantity.

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Tables

Table 1. Two years variance analysis of effects of covering times and kinds on infected strands.

Sources of difference	Df	SS	MS	F
Year	1	26279.209	26279.209	30.8244**
Replicate	14	48824.654	3487.475	4.0907**
Factor A (covering time)	1	656.384	656.384	0.7699ns
LA (year×covering time)	1	553.053	553.053	0.6487ns
Factor B (kind of cover)	4	25138.089	6284.522	7.3715**
LB (year×kind of cover)	4	7507.174	1876.793	2.2014ns
AB (covering time×kind of cover)	4	1408.867	352.217	0.4131ns
LAB (year×covering time×kind of cover)	4	898.496	224.624	0.2635ns
Experiment error	126	107420.586	852.544	-
Total	159	218686.511	-	-

**=significant difference at 1% probability level.
ns=no significant difference.

Table 2. Two years variance analysis of effects of covering kinds and times on infected fruits.

Sources of difference	Df	SS	MS	F
Year	1	41984.451	41984.451	54.5126**
Replicate	14	50893.682	3635.263	4.7200**
Factor A (covering time)	1	335.074	335.074	0.4351
LA (year×covering time)	1	90.689	90.689	0.1178
Factor B (kind of cover)	4	7345.653	1836.329	2.3843
LB (year×kind of cover)	4	2548.653	635.163	0.8273
AB (covering time×kind of cover)	4	679.602	169.901	0.2206
LAB (year×covering time×kind of cover)	4	1474.673	368.667	0.4787
Experiment error	126	97042.503	770.179	-
Total	159	202394.664	-	-

**=significant difference at 1% probability level.

Figures

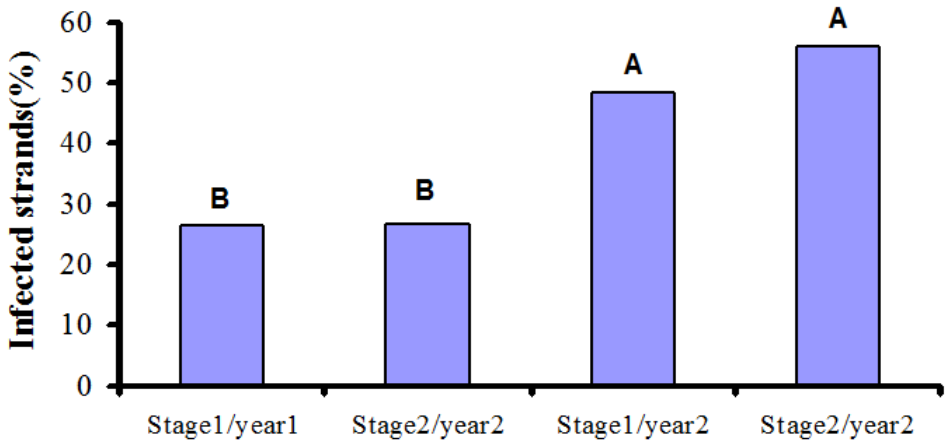

Fig. 1. Effect of year and time of covering on bunch infection.

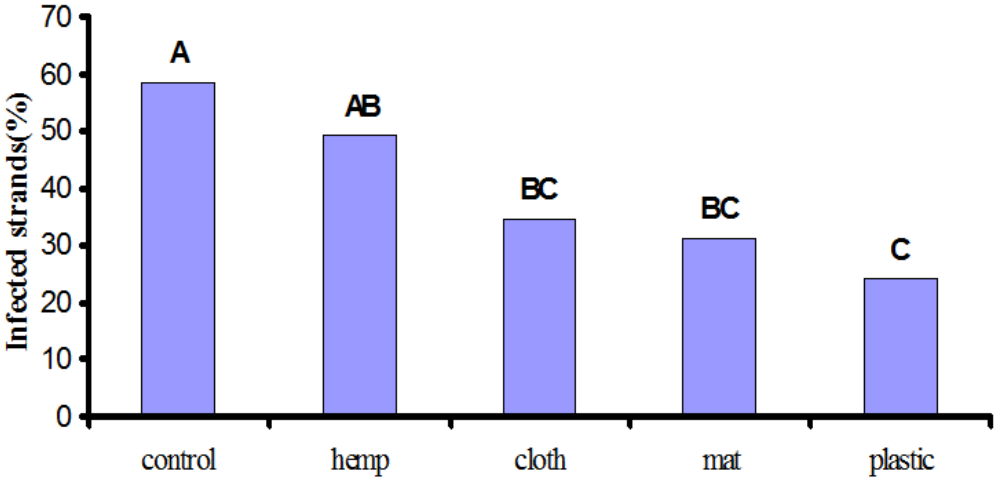

Fig. 2. Effect of kind of cover on bunch infection.

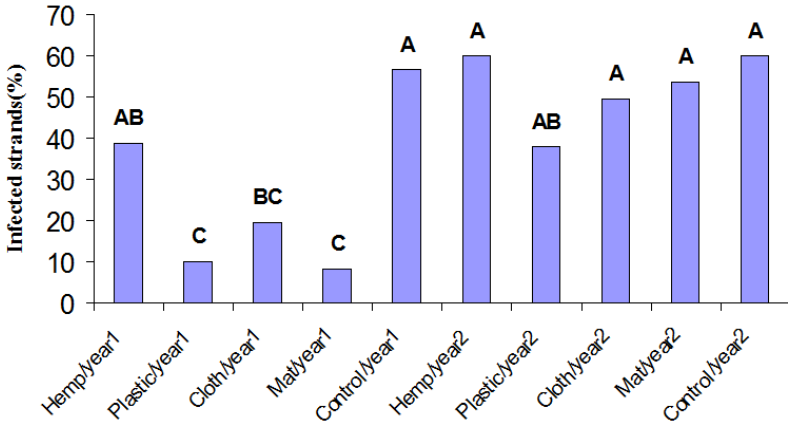

Fig. 3. Effect of year and kind of cover on bunch infection.

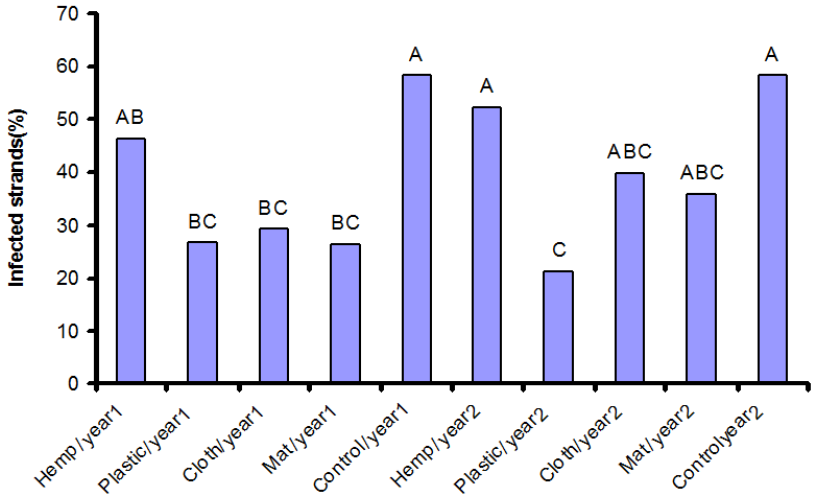

Fig. 4. Effect of kinds and times of covering on bunch infection.

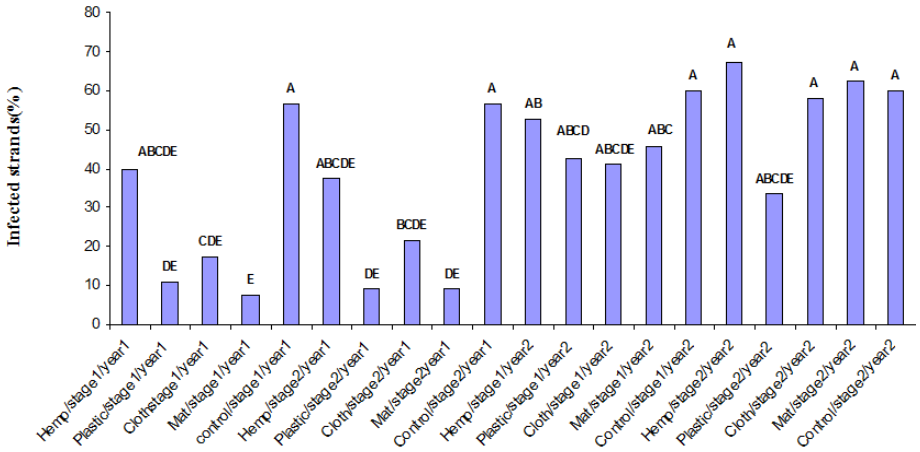

Fig. 5. Reciprocal effects of year/time/kinds of covers on bunch infection.

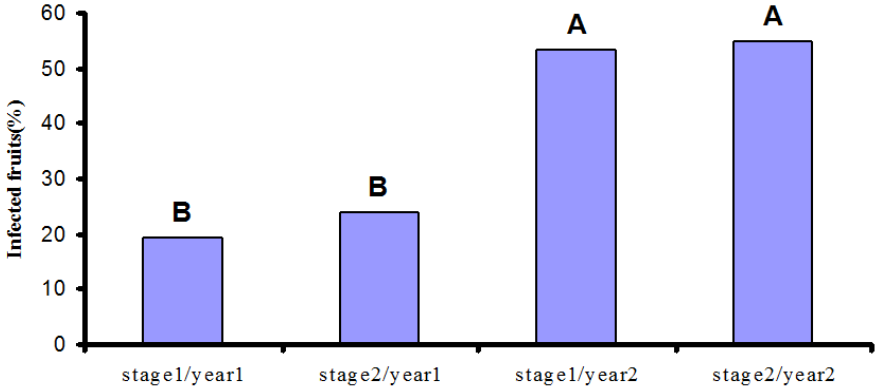

Fig. 6. Effect of year per time of covering on fruit infection.

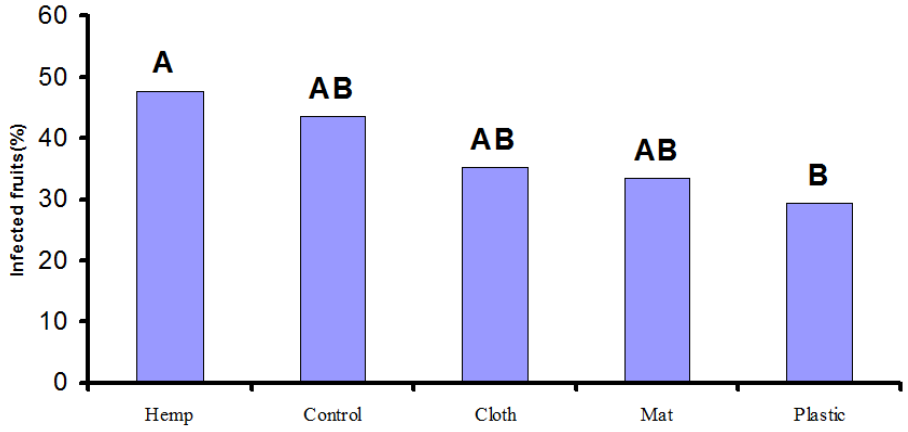

Fig. 7. Effect of kind of cover on fruit infecton.

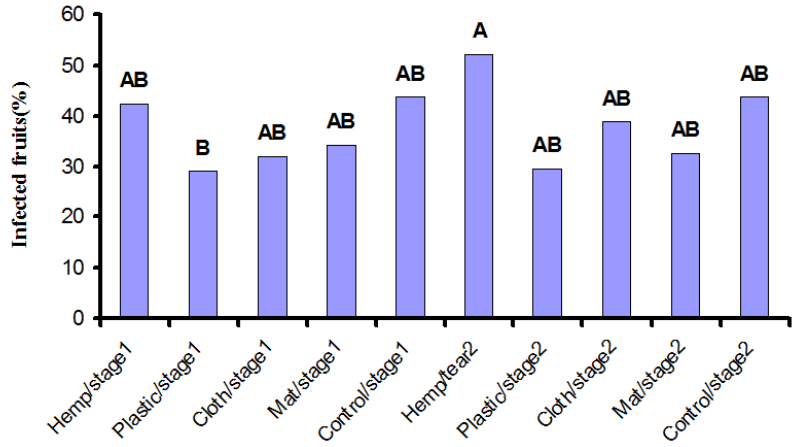

Fig. 8. Effect of time and kind of cover on fruit infection.

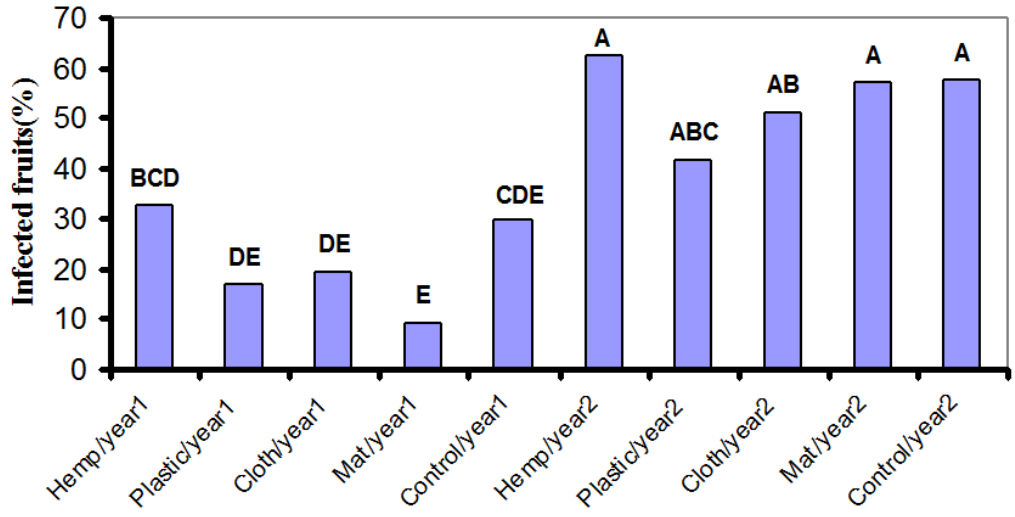

Fig. 9. Effect of year and kind of covers on fruit infection.

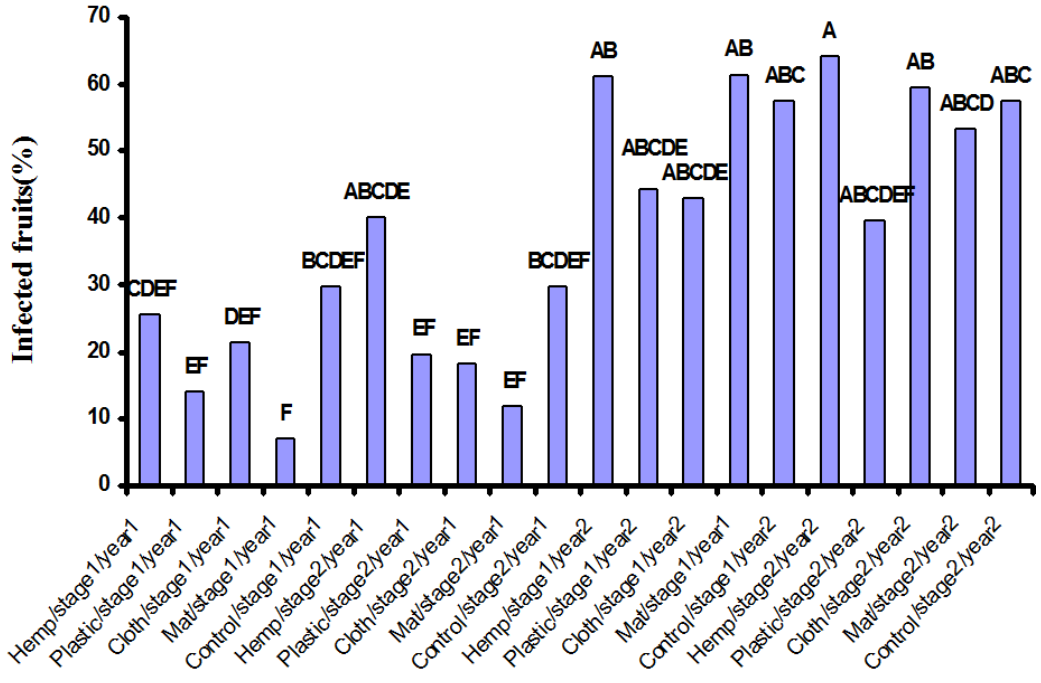

Fig. 10. Reciprocal effects of year/time/kind of covering on fruit infection.