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5th Date Palm Conference

Date Palm Friends Society

DPGN
Date Palm Global Network

PROCEEDINGS OF THE
FIFTH INTERNATIONAL DATE PALM CONFERENCE

EDITORS
ABDELOUAHHAB ZAID | GHALEB ALI ALHADRAMI

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FOREWORD

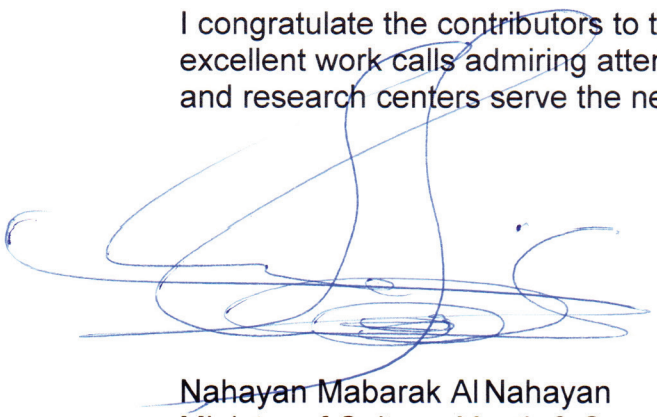
I am pleased to introduce these proceedings for the Fifth International Date Palm Conference that was held under the High Patronage of His Highness Sheikh Khalifa Bin Zayed Al Nahyan, President of United Arab Emirates. The conference was organized by the United Arab Emirates University in collaboration with the Ministry of Presidential Affairs, the Khalifa International Date Palm Award, the Date Palm Global Network, and the Date Palm Friends Society.

The conference was attended by UAE government officials, ambassadors representing their nations in the UAE, members of international educational and research institutions, scientists, technicians, and private date growers. More than 400 participants from 42 countries attended the conference.

The conference sought three objectives: to provide an opportunity for updating scientific information on different aspects of date palm production, propagation, protection, and marketing; to compare the recent experiences in the United Arab Emirates with those of other date-growing countries; and to foster international technical cooperation on different aspects of the date palm production chain.

The research presented at this conference, as published in these proceedings, demonstrates the value of careful scholarship and the creative imagination to social and economic development. The research enlarges our understanding of the significance of the date palm in the 21st century and encourages regional and international collaboration. The research will enhance the economic value of the date palm and will promote its role in agricultural development. These proceedings will surely help to attract new generations of scientists, technicians, and entrepreneurs to the field of Date Palm Research and Development.

I congratulate the contributors to these proceedings and am confident that their excellent work calls admiring attention to the important ways that universities and research centers serve the needs of their communities.



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PREFACE

The Proceedings of the Fifth International Date Palm Conference is published by Khalifa International Date Palm Award. Keynote speakers and authors of selected contributed oral presentations were given the opportunity to submit a manuscript for publication.

These manuscripts were reviewed by the conference editors and members of the editorial board. Only those papers judged suitable for publication following the authors' consideration of reviewer suggestions appear in this Proceedings of the Fifth International Date Palm Conference.

Khalifa International Date Palm Award acknowledges and appreciates
the contribution of all authors, editors and reviewers.

Editorial Board.

Prof. Abdelouahhab Zaid and Prof. Ghaleb Ali Alhadrami

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Current Status of Date Palm Cultivation

Evaluation of some date palm cultivars grown under toshky conditions

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ABSTRACT

This study was carried out during two successive seasons of 2011 and 2012 on some date palm cultivars grown under the conditions of Toshky region. Seven date palm cultivars were evaluated and classified to two groups: dry date palm cultivars (Sakkoty, Bartamoda, Gondela, Malkaby and Balady [Maghal]) and soft date palm cultivars (Barhee and Sokkary). Sakkoty and Bartamoda (dry date palm cultivars) and Barhee (soft date palm cultivar) gave the highest number of leaves per palm/year, while Malakaby (dry date palm cultivar) and Sokkary (soft date palm cultivar) gave the highest number of leaflet per leaf in both seasons. Bartamoda (dry date plm cultivar) and Sokkary (soft date palm cultivar) gave the highest yield, fruit weight and flush weight in the two seasons. Balady [Maghal] (dry date palm cultivar) and Barhee (soft date palm cultivar) showed higher moisture content (%) in both seasons. Bartamoda (dry date palm cultivar) and Sokkary (soft date palm cultivar) gave the highest soluble solids content and total sugars (%) while Gondela (dry date palm cultivar) and Sokkary (soft date palm cultivar) gave the highest reducing sugars (%) in the two seasons. Evaluation study revaluated that Sakkoty and Bartamoda were the best dry date palm cultivars. Wherever, Sokkary cultivar was the best soft date palm cultivars growing under Toshky conditions.

Key words: Date palm - Evaluation - Cultivar – Soluble Solids Content.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is major and most important fruit crop grown in Toshki region, where high temperature and poor soil quality profound. It plays a great socioeconomic important role and is widely used for food and many other commercial purposes.

The most important commercial date palm cultivars (Sakkoty, Bartamoda, Gondela, Malkaby and Balady) cover a great proportion of the Aswan cultivation. In addition, there are few date palm cultivars (Sokkary and Barhee) showed very good qualities in such location.

Date palm cultivars are of three main types according to its fruit moisture content, i.e. Soft, Semi-dry and dry cultivars (Selim *et al.,* 1968). Date palm trees could grow under unfavorable conditions where many of other fruit species could not grow. Date palm is the most common fruit tree grown in semiarid and arid- regions it plays an important role in the protection of interplant cropping systems and the stabilization of the ecological system (Hasnaoui et al. 2011). For this reason date palm is considered one of the suitable trees which could be cultivated in the new reclaimed desert regions. Date palm fruits are one of the most important export fruit crops in Egypt, where they are harvested and marketed at three stages of their development. The three stages are khalal (bisr), rutab and tamar (Kassem 2012). The chemical composition of dates is variable due to various factors such as cultivar, region, climate, amount of fertilization and type of cultural practices (Al-Rawahi et al. 2005).

The differences between cultivars or strains of date palm may be due to either cytological difference between them, or to the more-genotypes that produced from seeds, (Al-Dose et al. 2001 and Al-Salih & Al-Sheik Hassain, 1980). Morphological characters for leaves and fruits could be used in identification and description of date palm cultivars. Vegetative growth

parameters represented 28% of variance between date palm cultivars. Also, spathe, length and weight of spathe, length of stand and number of flowers on stand represented 41% from the variance among date palm cultivars. Fruit properties such as fruit weight, length, size, total sugars, SSC, tannins and fibers content represented 31% from variance. (Ismail et al. 2008 and Rizk et al. 2007). Physical and chemical characteristics of date palm fruits depending on up cultivars and environmental conditions (Mohamed et al. 2004).

Moreover, the chemical compositions of 8 date's cultivars from different areas of Upper Egypt were evaluated. Total sugars content ranged from 73.65% to 81.77% for dry cultivars and from 75.10% to 87.27% for semi-dry cultivars. Non reducing sugars (41.85%-46.52 %) were the dominant sugars of dry cultivars, while reducing sugars (71.83%- 79.08%) were present in high amounts in the semi-dry cultivars (Youssef et al 1999).

The aim of this work is to survey and evaluation of date palm cultivars under toshky conditions to know the suitable cultivars to grow and product under these conditions.

MATERIALS AND METHODS

The present study was carried out during the two successive seasons of 2011 and 2012 on evaluate vegetative growth, physic al and chemical fruit properties of some date palm cultivars grown under the conditions of Toshky, Aswan Governorate, Egypt. These cultivars are classified and nominated according to their moisture content into two groups as follows: a- dry date palm cultivars (Sakkoty, Bartamoda, Gondela, Malkaby and Balady) b- soft date palm cultivars (Sokkary and Barhee).

Each cultivar was represented by 6 palms in three replication. The palms of about ten years old grown on sandy soil. The experimental palms were propagated with tissue culture and irrigated by (650 ppm).The palms were similar in vigor and received the same orchard management. The inflorescences of the trees under this study were manually pollinated by one source of pollen. The yield of Sakkoty, Bartamoda, Gondela, Malkaby and Balady trees (dry date palm cultivars) were harvested through the first half of September, while Sokkary and Barhee (soft date palm cultivars) were harvested through the first of August and the first half of July, respectively during the fruit ripening stage. Three fully grown leaves per tree were examined for leaf length, number of leaflet per leaf, leaf base zone width, spine zone length and trunk diameter.

For fruit properties fifty fruits were randomly taken from each palm. Physical properties of fruits were determined at the peak of the "full color" stage. Average weight of fruit, flesh and seed and fruit dimensions. Chemical properties of fruit juice (ten date fruits from each palm tree were cut into pieces after omitting seeds. 50 gram portion was blended in 100 ml distilled water using special electric mixer, then filtered and the filtrate was taken for analysis) were determined as outlined by A. O. A. C. (1995) including moisture percentage, sugars (total, reducing and non-reducing sugars) and soluble solids content (SSC) in fruit juice was estimated using a hand refractometer.

All collected data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980). Treatment means were compared using the Duncan least significant range (Duncan, 1955) at the 5 percent level of significance in both seasons of experimentation.

Date palm fruits obtained from the present study were numerically ranked according to some of the points (units) based on the rank number given to each fruit chemical and physical property. The maximum comprehensive total of points was defined to be a hundred points (Mousa, 1981 and Bakr *et al.,* 1985). The hundred points were distributed as follows: 20 points for the average fruit yield per palm, 20 the average bunch weight, 20 the average fruit weight, 20 the average total sugars, 10 average leaf length and 10 the average fruit flesh weight.

Generally, the fruits of a cultivar that surpass other fruits in a fruit parameter such as average fruit weight will be assigned the maximum points assigned to this parameter. For example, if the cultivar (X) recorded the maximum for fruit weight, it will take 10 points or units, while the fruits of the others will take points relative to this maximum. This can be calculated according to the following equation:

Points of cultivar (a) = (fruit weight of (a)/fruit weight of (X) * 10

Thus, fruits of all studied cultivars can be ranked in the same way. This can be followed for the measurements obtained for the physical and chemical properties of studied fruits.

RESULTS AND DISCUSSIOND

I- Vegetative growth parameters:

Data presented in Table (1) show the average number of leaves per palm/year, leaf length, number of leaflet per leaf, leaf base zone width, spine zone length and trunk diameter during 2011 and 2012 seasons.

1- Number of leaves per palm/year:

Results of the two seasons revealed that, in respect to dry date palm cultivars, Sakkoty and Bartmoda cultivars gave the highest number of leaves per palm/year in the two seasons. Regarding to soft date palm cultivars there were no significant differences between Barhee and Sokkary cultivars during 2011 and 2012 seasons.

2- Leaf length (m):

It is clear from data in Table (1) that no significant differences between dry date palm cultivars but Gondela and Mlakaby cultivars gave the highest leaf length as compared with other cultivars in the first and second seasons, respectively. Barhee (soft date palm cultivar) gave the highest leaf length as compared with Sokkary cultivar in the first season but no significant differences between them in the second season n this respect.

El-Bakr (1972), Sewy and Karama date palm cultivars lie under the group of short leaf cultivars. Oawshingbeat, Tagtaggt and Ghazal strains lies under the medium leaf cultivars, while the Freahy date palm cultivar lies under long leaf cultivars. Osman (2007) found that leaf length of Sakkoty date palm cultivar ranged between 206 - 216 cm. Rizk and Nahed, (2006) found that the Freahy cultivar the highest significant values regarding leaf length.

3- Number of leaflets per leaf:

Data presented in Table (1) indicated that significant differences in number of leaflets per leaf among the studied cultivars. Concerning dry date palm cultivars, Malakaby followed by Gondela cultivars gave the highest number of leaflet per leaf as compared with other dry date palm cultivars in the first and second seasons. Regarding soft date palm cultivars, Sokkary cultivar gave higher number of leaflets per leaf than Barhee cultivar in the two seasons.

In the respect, Abdella (1979) found that number of leaflets per leaf was greatest in Helwa (114-116 leaflets) and Sayer (97 leaflets). Leaflets of Samany, Barhee and Sayer were longer and narrower (53-55 cm in length and 2.3-2.5 cm in width)

4- Trunk dia meter (m):

Results in Table (1) indicated that there is no significant difference in trunk diameter among the studied cultivars in both seasons. Malakaby (dry date palm cultivar) gave the highest trunk diameter as compared with other cultivars, while Sokkary (soft date palm cultivar) gave higher trunk diameter than Barhee cultivar in both seasons.

5- Leaf base zone width (cm):

Data presented in Table (1) clearly indicated that the leaf base zone width was not significant among the studied cultivars. Malakaby cv. gave the highest width of leaf base zone as compared with other dry date palm cultivars in the first and second seasons. While Barhee (soft date palm cultivar) gave width of leaf base zone higher than Sokkary cultivar in both seasons.

6- Spine zone length (m):

Noticeable is that spine zone length was not significant differences between dry date palm cultivars in both seasons. Gondela cultivar gave the highest spine zone length as compared with other cultivars in the two seasons. However, data show that spine zone length significant differences between soft date palm cultivars in the second season only. Barhee cultivar gave higher spine zone length than Sokkary cultivar in the second season, Table (1).

Rizk and Nahed, (2006), found that spine zone length, Ghazal and Karama strains gave the least values. Regarding leaf base zone width, the Freahy cultivar significantly gave the least values, whereas the Oashingbeal gave the highest significant values.

II- Yield per palm (kg):

Data presented in Table (2) indicated that no significant differences on yield per palm among the studied cultivars. Regarding dry date palm cultivars, Bartamoda cultivar gave the highest yield as compared with other dry date palm cultivars in both seasons. Sokkary (soft date palm cultivar) gave the highest yield per palm in the first season. While, Barhee (soft date palm cultivar) gave the highest values in the second season.

According to Rizk and Nahed, (2006) found that Sewy cultivar gave the highest yield followed by the strain Ghazal, while the strains Karama and Tagtaggt showed the lowest significant values in both seasons.

III- Bunch weight (kg):

Concerning the bunch weight, the obtained results indicated that there were significant differences between studied cultivars during the first season only. Bartamoda cultivar gave the highest bunch weight, while, Balady (Maghal) gave the lowest bunch weight as compared with other dry date palm cultivars. In the second season there were no significant differences among all dry date palm cultivars in bunch weight. Regarding soft date palm cultivars there were no significant differences in this respect in both seasons, Table (2).

These results are in agreement with what El-Makhtoune and Abdel-Kader (1990), mentioned in this regard, they stated that the average bunch weight ranged from 4.22 to 34.40 kg according the date palm cultivar.

IIII- Fruit physical and chemical properties:

A- Fruit physical properties:

1- Fruit length (cm):

Concerning the fruit length in table (2) the results indicated that, there were significant differences among

the studied cultivars in both seasons. Gondela (dry date palm cultivar) gave the highest fruit length in the two seasons. However, Balady (Maghal) gave the lower values in fruit length as compared with other dry date palm cultivars in the two seasons of this study. On the other side, there were no significant differences between soft date palm cultivars in both seasons in this respect.

Generally, these results are in harmony with those obtained by Habib et al. (1984), Hussein et al. (1984), Al-Ghamdi (1996) and Hussein et al. (2001), they noticed that highly significant differences in fruit length among cultivars in most of fruit characteristics.

2- Fruit diameter (cm):

It is noticed from the results in Table (2) that during two seasons, the fruit diameter exhibits similar trend as the fruit length.

These results agreed generally with those found by Hussein and Hussein (1982) and Nour et al. (1986) on dry varieties under Aswan conditions, while Hussein et al. (2001) on different varieties under conditions of Siwa Oasis.

3- Fruit weight (g):

Regarding the fruit weight in Table (2) the results indicated that there were significant differences among the studied cultivars. The higher values were observed with Bartamoda as compared with comparable values obtained from other dry date palm cultivars in both seasons. On the other side, Sokkary (soft date palm cultivar) gave the highest fruit weight as compared with Barhee cultivar in the two seasons.

Rizk et al. (2006) reported that the maximum values of physical characteristics of fruits were found in Siwy cultivar, while the lowest values were found in Freahy cultivar. In this concern, Selim et al. (1968) found variable results dealt with Siwy cultivar.

4- Flesh weight (g):

Concerning the flesh weight, the results indicated that it was significant differences among studied date palm cultivars during the two seasons. Bartamoda cultivar gave the highest flesh weight as compared with other dry date palm cultivars in both seasons. On the other hand, Sokkary (soft date palm cultivar) gave the highest flesh weight than Barhee cultivar in the two seasons, Table (2).

5- Seed weight (g):

The results in Table (2) indicated that significant differences in seed weight among the studied cultivars in both seasons. Balady (Maghal) gave the lowest weight of seed as compared with other dry date palm cultivars in the two seasons. While seed weight of soft date palm cultivars did not significantly differences in both seasons of this study. However, Barhee cultivar gave the lowest seed weight as compared with Sokkary cultivar in the two seasons.

Rizk et al. (2006), reported that the highest values of seed weight was found in Siwy cultivar, while the lowest values were found in Freahy cultivar. In this concern, Selim et al. (1968) found variable results dealt with Siwy cultivar.

6- Flesh percentage (%):

Data presented in Table (2) indicated that significant differences in flesh percentage among the studied cultivars in the second seasons only. Bartamoda (dry date palm cultivars) gave the highest flesh percentage, while Balady (Maghal) cultivar show the lower values in flesh percentage as compared with other dry date palm cultivars in the second season. On the other hand, no significant differences between the two soft date palm cultivars in this respect during 2011 and 2012 seasons.

B- Fruit chemical properties:

1- Moisture content (%):

Data presented in Table (3) indicated that significant differences in moisture percentage among dry date palm cultivars in the first season only and soft date palm cultivars in the two seasons. Concerning dry date palm cultivars, moisture percentage of fruits ranged from 15 to 19.98%. While soft date palms cultivars, moisture percentage of fruits ranged from 42.73 to 63.67%. Selim et al. (1968), found variable results dealt with Siwy cultivar.

2-Soluble solids contents (SSC %):

The results in Table (3) indicated that significant differences in soluble solids content among the studied cultivars. Concerning dry date palm cultivars Bartamoda cultivar gave the highest soluble solids content, while, Balady cultivar (Maghal) gave the lowest soluble solids content as compared with other dry date palm cultivars. On the other side, significant differences were found between soft date palm cultivars. Sokkary cultivar gave higher percentage of soluble solids content than Barhee cultivar in the first and second seasons.

Generally, differences between the all cultivars were significant; these findings are in agreement with those of Selim *et al.,* (1968) who reported that total soluble solids of dry date fruits ranged from 45-60%. While Hussein and Hussein (1982) reported that the total soluble solids of Sakkoty fruits ranged between 64.20 and 70.30. Nour *et al.,* (1986) found that total soluble solidsof some dry date palm fruits ranged between 54 and 63.1%. Al-Ghamdi (1996) showed that significant differences among cultivars in total soluble solids. Hussein et al. (2001) who reported that total soluble solids of dry date fruits ranged from 13.7-19.8%,

semi-dry date palm cultivars ranged from 22.67-28.83% and soft date palm cultivars ranged from 41.50-66.10%.

3- Total sugars (%):

Data presented in Table (3) indicated that significant differences in total sugars content among the studied dry and cultivars in the two seasons. Bartamoda (dry date palm cultivars) gave the highest total sugars content as compared with other dry date palm cultivars. Concerning soft date palm cultivars, Sokkary cultivar gave the highest total sugars content than Barhee cultivar in the first and second seasons.

Many other studies reported that total sugars content of fruit in some of date palm cultivars on dry weight basis. Hussein and Hussein, (1982) reported that total sugars content of fruit ranged between 55.99 to 58.89% for Sakkoty fruit. Hussein et al. (2001) reported that total sugars of fruit ranged between 51.45-56.10%, 53.64-56.50% and 36.30-60.20% of (dry date palm cultivars), (semi-dry date palm cultivars) and (soft date palm cultivars), respectively.

4- Reducing sugars (%):

The results in Table (3) indicated that significant differences reducing sugars among the studied cultivars. Fruits of Gondela (dry date palm cultivars) gave the highest reducing sugars content as compared with other dry date palm cultivars in both seasons. However, Sokkary cultivar (soft date palm cultivar) gave reducing sugars content higher than Barhee cultivar in the first and second seasons.

5- Non-reducing sugars (%):

Concerning the non-reducing sugars content, the obtained results indicated that there were significant differences among dry date palm cultivars in the first and second seasons and between soft date palm cultivars in the first season only. fruits of Bartamoda cultivar (dry date palm cultivars) contain the highest non-reducing sugars content as compared with other dry date palm cultivars in both seasons. While fruits of sokkary cultivar recorded non-reducing sugars higher than Barhee cultivar in the first season only but no significant differences between the two cultivars were found in the second season in this respect.

V- The final evaluation:

Data in table (4) clearly indicated that Sokkary (soft date palm cultivars) was recorded the highest units in bunch weight and yield, followed by Barhee (soft date palm cultivars), while Gondela (dry date palm cultivars) was recorded the lowest units in this respect. Concerning total sugars percentage, Bartamoda (dry date palm cultivars) recorded the highest units in total sugars percentage, but Barhee (soft date palm cultivars) was recorded the lowest units in this respect. Regarding fruit weight, Bartamoda followed by Gondela (dry date palm cultivars) recorded the highest units in fruit weight, while Sakkoty (dry date palm cultivars), was recorded the lowest units in fruit weight.

On the other side, leaf length, Barhee cultivar was recorded the highest units in leaf length followed by Sokkary (soft date palm cultivars). However, Bartamoda (dry date palm cultivars) followed by Barhee (soft date palm cultivars) was recorded the highest units in flesh percentage. Finally, Sokkary and Barhee (soft date palm cultivars) were the best soft date palm cultivars and the addition of common of all dry date palm cultivars under conditions of Toshky.

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Tables:

Table (1): some vegetative growth parameters of the date palm cultivars studied at Toshky during 2011 and 2012 seasons.

Cultivars	Number of leaves/palm	Leaf length (m)	Number of leaflet/leaf	Trunk diameter (m)	Leaf base zone width(cm)	Spine zone length(m)
The first season						
Dry cultivars:						
Sakkoty	34.67 A	2.92 A	124.3 B	0.9733 A	12.00 A	1.00 A
Bartamoda	33.00 AB	2.98 A	129.0 B	0.9833 A	12.02A	0.97 A
Gondela	30.33 C	3.07 A	130.3 A	0.9600 A	12.33 A	1.10 A
Malakaby	30.67 BC	3.00 A	133.0 A	1.0233 A	12.33 A	1.07 A
Balady	30.61 C	2.97 A	130.1 A	0.9700 A	12.01 A	0.99 A
Soft cultivars:						
Sokkary	90.00 A	4.07 B	214.0 A	1.6500 A	18.00 B	1.30 A
Barhee	90.33 A	4.17 A	194.0 B	1.6000 A	20.00 A	1.50 A
The second season						
Dry cultivars:						
Sakkoty	35.00 A	3.00 A	124.33 C	0.9766 A	11.00 A	1.00 A
Bartamoda	34.00 AB	3.01 A	127.70 BC	0.9733 A	11.67 A	0.98 A
Gondela	31.33 BC	2.99 A	130.00 AB	0.9800 A	11.67 A	1.07 A
Malakaby	30.00 C	3.10 A	133.67 A	1.0300 A	13.00 A	0.95 A
Balady	30.09 C	3.00 A	127.00 BC	0.9700 A	11.69 A	0.97 A
Soft cultivars:						
Sokkary	90.00 A	4.10 A	212.67 A	1.64 A	19.00 A	1.32 B
Barhee	90.33 A	4.13 A	196.00 B	1.59 A	20.00 A	1.52 A

Table (2): some fruit physical properties of the date palm cultivars studied at Toshky during 2011 and 2012 seasons.

Cultivars	Yield (kg/ palm)	Bunch weight (kg)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Flesh weight (g)	Seed weight (g)	Flesh percentage
The first season								
Dry cultivars:								
Sakkoty	69.03 A	7.7 AB	1.66 C	1.78 C	7.40 C	6.43 C	1.05 B	87.00 A
Bartamoda	78.03 A	8. 7 A	2.02 B	2.02 B	11.29 A	10.81 A	1.02 B	95.77 A
Gondela	60.03 A	6. 7 B	2.31 A	2.31 A	10.81 A	9.53 B	1.31 A	88.40 A
Malakaby	63.00 A	7.0 AB	2.12 AB	2.12 B	10.06 B	8.59 B	1.31 A	85.30 A
Balady	56.40 A	4.70 C	1.54 D	1.69 D	4.70 D	3.70 D	1.00 B	78.72 A

Cultivars	Yield (kg/ palm)	Bunch weight (kg)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Flesh weight (g)	Seed weight (g)	Flesh percentage
Soft cultivars:								
Sokkary	96.00 A	10.3 A	3.80 A	3.13 A	20.10 A	17.81 A	2.12 A	88.67 A
Barhee	92.97 A	10.3 A	3.88 A	2.77 A	15.91 B	14.37 B	1.45 A	90.33 A
The second season								
Dry cultivars:								
Sakkoty	72.00 A	8.0 A	1.73 C	1.73 C	7.42 C	6.19 C	1.15 AB	83.53 B
Bartamoda	78.03 A	8.7 A	1.97 B	1.97 B	11.54 A	10.63 A	0.99 B	93.43 A
Gondela	63.00 A	7.0 A	2.35 A	2.35 A	11.00AB	9.89 AB	1.27 A	88.63 AB
Malakaby	72.00 A	8.0 A	2.04 B	2.04 B	9.63 B	8.53 B	1.36 A	85.87 AB
Balady	64.80 A	5.4 A	1.66 D	1.60 D	4.77 D	3.74 D	1.03 C	78.41 C
Soft cultivars:								
Sokkary	102.7 A	10.67 A	3.77 A	3..17 A	20.50 A	18.11 A	2.39 A	87.53 A
Barhee	110.3 A	10.3 A	3.87 A	3.00 A	15.93 B	14.45 B	1.48 A	89.97 A

Table (3): some fruit chemical properties of the date palm cultivars studied at Toshki during 2011 and 2012 seasons.

Cultivars	Moisture content (%)	TSS %	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)
The first season					
Dry cultivars:					
Sakkoty	18.00 A	59.60 C	55.43 C	24.87 B	30.57 B
Bartamoda	15.00 B	62.87 A	59.33 A	24.80 B	34.53 A
Gondela	19.00 A	58.83 C	55.00 C	26.87 A	28.13 C
Malakaby	18.00 A	61.27 B	58.13 B	23.90 C	34.23 A
Balady	19.98 A	53.13 D	50.00 D	23.00 D	27.00 D
Soft cultivars:					
Sokkary	42.33 B	49.40 A	40.80 A	27.50 A	13.30 A
Barhee	63.67 A	44.00 B	30.00 B	20.90 B	9.10 B
The second season					
Dry cultivars:					
Sakkoty	17.77 A	59.27 C	55.47 B	24.83 B	30.63 B
Bartamoda	15.00 A	63.27 A	59.13 A	24.73 B	34.40 A
Gondela	18.77 A	58.93 C	55.10 B	26.27 A	28.83 C
Malakaby	18.33 A	61.00 B	56.57 B	23.83 C	32.73 A
Balady	19.90 A	52.17 D	51.12 C	23.12 D	28.00 C

Cultivars	Moisture content (%)	TSS %	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)
Soft cultivars:					
Sokkary	46.67 B	49.50 A	41.53 A	27.67 A	13.87 A
Barhee	63.67 A	44.43 B	30.60 B	20.83 B	9.77 A

Table (4) The evaluation units of some date palm cultivars grown under Toshky conditions.

Characters	Bunch weight (kg)	Palm yield (kg)	Total sugars (%)	Fruit weight (g)	Leaf length (m)	Flush percentage	General evaluation
Units specified	20	20	20	20	10	10	100
Dry cultivars:							
Sakkoty	14.9	14.9	18.6	6.4	7.1	9.0	71.0
Bartamoda	16.5	16.8	19.9	9.9	7.2	10.0	80.3
Gondela	13.0	13.0	18.5	9.4	7.3	9.4	70.6
Malakaby	14.3	14.3	19.3	8.5	7.3	9.0	72.7
Balady	14.0	14.1	16.0	8.3	7.2	9.2	68.8
Soft cultivars:							
Sokkary	20.0	20.0	13.8	17.5	9.8	9.3	90.5
Barhee	19.7	19.7	10.2	13.7	10.0	9.5	82.8

Phyllotactic variability of some Algerian date palm varieties

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ABSTRACT

The phyllotaxis phenomenon for certain date-palm (Phœnix dactylifera L.) was not deeply and thoroughly studied. Indeed, our purpose aims to confirm some phyllotaxis hypothesis for seven Algerian date-palm varieties. Using an empirical method, we have studied the phyllotactic variability based on biometrical measures of date-palm trunk. The study shows that for the divergence angle, orthostical distance and parastichy's slope in each contact parastichy matters in their phyllotactic modeling. The results confirm an intravarietal difference in the phyllotaxis of the seven studied varieties. The particular concluded remark is that the thirteenth parastichy of Itima variety become orthostic, which give the specific aspect (overlaid leaves) on the corona.

Keywords: Phyllotaxis, Contact Parastichy, *Phoenix dactylifera*, Algerian date-palm.

INTRODUCTION

The date palm's phyllotactic systems did not receive enough attention for in-depth study of *Phœnix Dactylifera L.*, There are few studies treating this subject, especially for the local varieties in Arab countries. Our study comes to check some hypotheses about the determinants of the phyllotactic systems for seven varieties of Algerian date palms.

With an empirical method, we aim to show the variability in phyllotactic systems, relying on a biometric measurements on the trunk of the palm, considering so that the divergence angle, the distance between orthostics, and the parastichy's slope in each contact parastichy which matters in modeling phyllotactic systems. The results confirm an intervarietal difference in the phyllotatic structure of the studied varieties. The particular concluded remark is that the 13th parastichy of Itima variety become orthostic, which give the specific aspect on the corona.

Our research paper is structured as following. First, a brief look at the conception evolution in the theoretical research of phyllotaxis. We focus after that on the phyllotactic systems of the palm, particularly, of the date palm. At second, we explain the methodology as well as experimental and modeling method used. Finally, we present and discuss the most important results obtained.

An Overview of Theoretical Framework of the Phyllotactic Systems

The phyllotaxis is currently considered as a multi-disciplinary with different methodologies. Guerreiro (1995) deduced its applications as a physical system and mathematical framework used in modern theoretical studies of phyllotaxis. The origins of the theoretical studies are relates to the work of Arthur Church (1904) who framed and theorized the phyllotaxis with mathematical approach. He has relied on the previous famous works of Bravais brothers with their descriptive approach. Every research had treated the simplest pattern of phyllotaxis.

The beginning of the in-depth studies coincide with the studies of the apical meristems structure where with studying the activity of this latter, we can understand the leaves' positions on the stem. The Plantefol theory (1947) has given a comprehensive approach of the apical meristem activity.

In modern approaches, Roger Jean's works are considered as the leading thesis in the modern theory. He presented several mathematical models in the last half century, and in-depth studies in apical activity (Jean, 1983), methodological studies

used for plant biology and phyllotaxis (Jean, 1986) and some surveys in this area (Jean, 1995), and provided with Irvin Adler (Adler *et al.,* 1997) a historical study in phyllotaxis.

Modeling Systems for the Phyllotaxis of Date Palm

The first studies had focused on oil palm phyllotaxis, relying on the above-mentioned works. Among these studies, was the study of Henry (1955), using the Plantefol's model for his descriptive study of oil palm phyllotaxis (Elais geinesis) considering the unique helix hypothesis. Rees (1964) present an in-depth study on the role of organizing apical meristem on phyllotaxis formations for Elais geinesis. Thomas et al. (1969) and on the same species, propose the equivalent phyllotaxis index (EPI) to explain fronds position on oil palm's trunk.

The study of Ferry (1998) comes to highlight the date palm phyllotaxis (*Phoenix dactylifera* L.) and is considered as the first study of the impact of the leaves shape characteristics on its phyllotaxis system where it has concluded that there are several models of the date palm using various methods. In contrast, Elhoumaizi et al. (2002) present a geometrical study of phyllotaxis, defining the divergence angle between fronds and its role in phyllotaxis systems and the phyllotactic variability. Moreover, Dror and Shimshoni (2009) suggest a study of the reconstruction of three-dimensional phyllotactic system for the date palm using modern techniques of simulation.

METHODOLOGY

In this study, we have adopted a biometric approach and we have acquired 5 895 measures on seven (07) date palm varieties located in the region of Biskra (Ziban oasis) which is considered as the most important region of the palm in Algeria. Three palm trees were chosen for each variety. In each palm tree, we have relied on four different measurements on the trunk that reflect the fronds positioning relative to each other.

Our study tries to find the possible relationships to consider a conceptual phyllotactic structure proving the following two hypotheses:

Hypothesis 1: The phyllotactic structures of date palm varieties differ according to their measurements.

All measurements (described later) differ in the selected seven varieties in our experience, which confirms the variability in the phyllotactic structure of the date palm trunk.

Hypothesis 2: The Itima represents a special case according to the parastichy slope.

We have note in the studied phenomenon that the contact parastichy differs in its parastichy slope from one to another (from 13, 8, 5 and 3). Furthermore, only the 13th parastichy in the case of Itima turns into an orthostic.

Variables

The studied phenomenon depends on the variable of diversity of phyllotactic forms as the dependent variable describing our phenomenon. The numeric values for this variable reflect the order of studied varieties as follows:

1. Deglet-Nour
2. Ghars
3. Mech-Degla
4. Itima
5. Safraye
6. Zogar-Mogar
7. Tati-Bent-Nouh

While four parameters (measurements) taken as variables explains the diversity of phyllotactic forms. These parameters (measurements) are regrouped in two kinds of measures. The direct one including the distance between the orthostics and the helix height, and the indirect one which include the divergence angle and helix slope.

The distance between the orthostics (A): It is the vertical distance between the two fronds in a same parastichy (cm).

The helix height (B): Is the height of helical unit (cm).

The slope helix: Calculated by the distance between the orthostics (A) and the distance between the two fronds in same parastichy (F) expressing the relationship as following:

$$\alpha = \cos^{-1}(A/F)$$

The divergence angle: Calculated by the twice distance between the orthostics (A) and the radius (r) expressed as following:

$$\theta = A/r$$

The figure 1 shows the various measurements in our experience.

The Model

The nature of the dependent variable in this phenomenon (diversity of phyllotactic forms) is a qualitative and taken seven value reflect the seven varieties studied. We use for that the probabilistic modeling (the Logit Model) which as appropriate modeling kind for our variables case, presented by the following formula:

$$\Pr[E(Y = k|X_i)] = \alpha_i X_i + \epsilon$$

Where Xi indicates the above measurements, relying on two statistical tests, the adjusted correlation coefficient and of course using the null hypothesis test p-value.

RESULTS AND DISCUSSION

The experiment confirms null-hypothesis test where the model is represented in the following formula:

$$Y = \underset{(.001)}{-.038}\ \alpha - \underset{(.001)}{.028}\ \theta - \underset{(.005)}{.093}\ A - \underset{(.002)}{.005}\ B$$

; R2=0.97P<0.0001

This model is significant, with a strong correlation between the different variables (Xi) and diversity variable, and each variable separately has a very significant effect with lower p-value, which confirms the null-hypothesis for each variable (see Table 1).

The two first graphs (see Figures) show an inverse relationship between the divergence angle and slope helix (Figure 2) and as well as the distance between the orthostics and the slope helix (Figure 3).

The latter shows clearly the inverse relationship of the various helixes. We can even notice the discrepancy between the various helixes. As Figure 2 shows an exceptional gathering where the divergence angle and slope helix is a large, specific to Deglet-Nour variety where 8th parastichy have a great divergence angle and slope helix. To prove our first hypothesis that relies on the variability of the phyllotactic structures of date palm and from their significance of results, we suggest the graphics (Figure 4 and 5).

Based on the three basic parameters, we have a clear contrast between the various phyllotactic systems of studied varieties, which clearly illustrate the seven gatherings. Figure 4 shows the relationship between the divergence angle and the helix height. Three mixed overlapping gatherings are illustrated and the same for Figure 5, which shows the relationship between the orthostic distance and the helix height.

We can denote also in Figure 6, that shows the divergence angles by varieties, a relative variation for each variety. We can confirm the difference between phyllotactic structures in the studied varieties.

The Figure 7 comes to confirm our second hypothesis showing the slope helix for the studied varieties, and indicates that the fourth variety (Itima) is characterized by the presence of a vertical parastichy (90°), which represents the 13th parastichy in contrast of the rest.

This feature could be observed clearly in this variety where the 13th parastichy represent an orthostic on the trunk as well as on the corona, which make it distinct variety among others with regular spaces between parastichies on the corona that resulted from the vertical overlaying of fronds.

CONCLUSION

As result of this study, we can conclude that the phyllotactic systems of the date palm (*Phoenix dactylifera* L.) vary among varieties. The experience was conducted on seven Algerian date palm varieties. Through some biometric measurements of the phyllotaxis, the modeling has allowed us to show the variability in phyllotactic structures. While, this study calls for several perspectives including more varieties, relying on the palm corona, and focusing on the physiological aspects of apical meristem activity.

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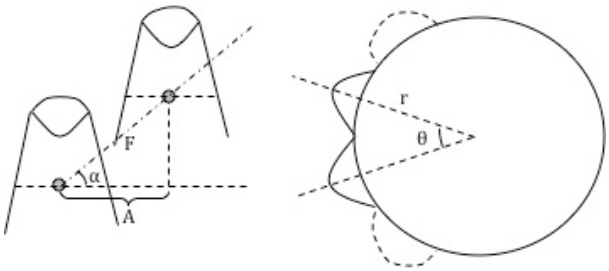

Figure 1. Representation of the used parameters

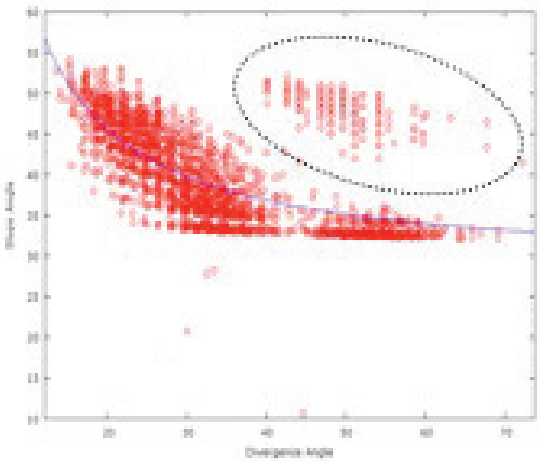

Figure 2.

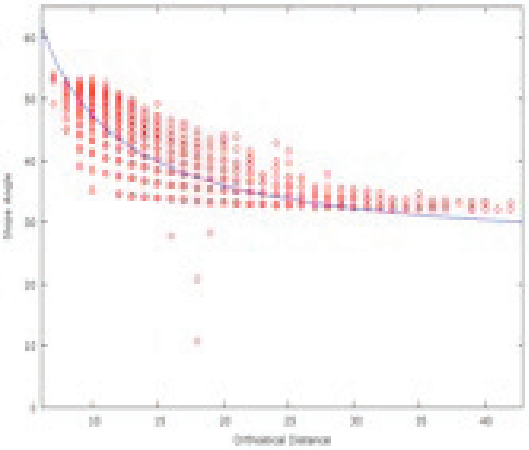

Figure 3.

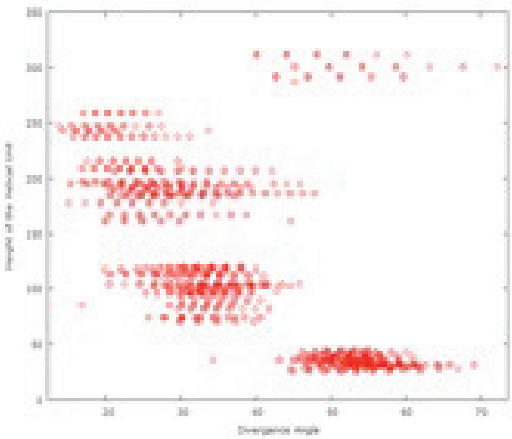

Figure 4.

Table 1. The Gretl output for the multinomial logit based on the experimental matrix

Logit Model, for n=5895 Observations, Dependant Variable = Var ∋ {1,2,3,4,5,6,7}

	Coefficient	Std. Error	t-Student	p-critique	
Height of Helical Unit (B)	-0,005127	0,0002242	-22,8661	<0,00001	***
Ortostical Distance (A)	-0,093862	0,0056447	-16,6284	<0,00001	***
Slope Angle (α)	-0,038982	0,0014744	-26,4376	<0,00001	***
Divergence Angle(θ)	-0,028524	0,0012188	-23,4023	<0,00001	***
Residuals Sum Square	1263,296			Sdt. Div. Reg.	0,563534
R2	0,9727S02			Adjusted R2	0,972675
F(5, 3978)	28349,72			p-Value (F)	0,000000
Schwarz Criteria	6770,991			Hannan-Quinn	6750,692

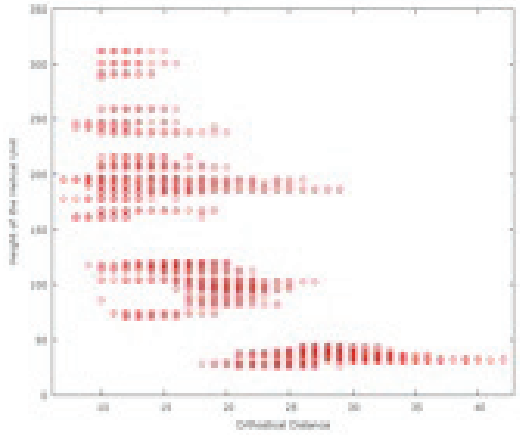

Figure 5.

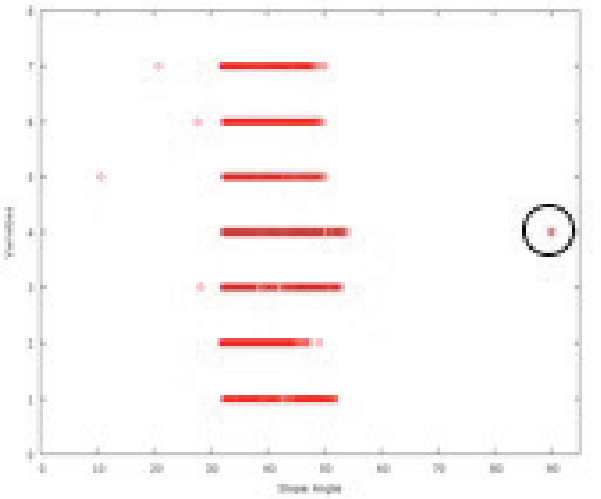

Figure 7.

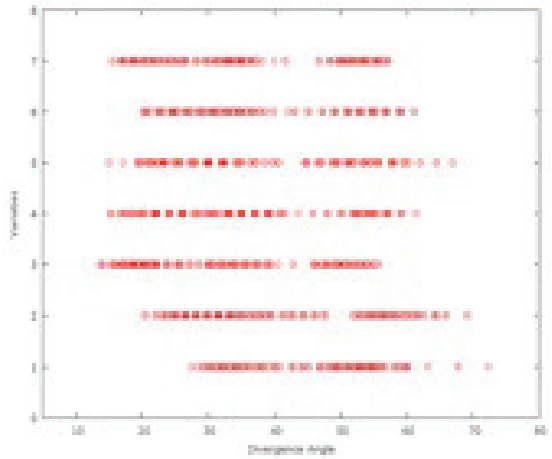

Figure 6.

Comparative study on date palm (*Phoenix dactylifera L.*) leaf spot fungal pathogens *Nigrospora oryzae* and *N. sphaerica*

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ABSTRACT

Date palm diseases is rising as an important concern during the last decades. The fungal pathogens of date palm are considered as the most serious problem causing significant reductions of growth, development, and production of date palm. Recently, in Iraq, several fungal pathogens have been isolated from heavily infected date palm leaves exhibiting symptoms of leaf spot; most abundantly, different species of Nigrospora. The present study was aimed at the characterization of these two Nigrospora species, isolated from different cultivars of date palm, based on morphological, molecular and pathological characteristics. In the current study, the identity of both Nigrospora species have been revealed to be as N. oryzae and N. sphaerica on the basis of their morphological characteristics and molecular analysis of the Internal Transcribed Spacer (ITS) region. Results showed that both pathogens were found to be true pathogens on different date palm cultivars. Compared to N. Sphaerica, N. oryzae was more aggressive on the following cultivars: Al-Sayer, Hillawi, Zahdi, Leloy and Kantar. After 30 days post-inoculation, the overall average lesion diameter was 1.85 cm in response to the artificial infection with N. oryzae, whereas infection with N. sphaerica produced 1.42 cm lesions. Al-Sayer cv. was the most susceptible, among the tested cultivars to both Nigrospora species, the lesion diameter was 2.50 cm, in contrast with cv. Leloy, 1.10 cm, which showed the lowest level of susceptibility . The extracellular enzymatic activity of both pathogens revealed that N. oryzae surpassed N. sphaerica in the production of cellulase and protease enzymes; whereas, lipase enzyme activity was absent in both fungi. The high enzymatic activity and virulence of N. oryzae on different date palm cultivars were approved in contrast with the species of N. sphaerica.

Keywords: Date palm, Enzyme activity, Leaf spot disease, Nigrospora oryzae, Nigrospora sphaerica

INTRODUCTION

Date palms (*Phoenix dactylifera* L.) are monocotyledon, dioecious plants, and one of the most cultivated palms around the world (Abass, 2013a). Date palm trees are cultivated in different regions worldwide, especially in Middle East, North Africa, Central and North America, Southern Europe, Pakistan and India (Zaid, 2002; Alshahib and Marshall, 2003). World production of date is estimated to exceed 7.5 million tons in 2009; the Arabian Peninsula contributes over one third of world total dates production (FAO, 2011).

Dates are well known as a good source of energy attributed to their rich content of nutrients, mostly carbohydrates and dietary fibre, certain essential vitamins and minerals

such as iron, potassium, calcium and low level of sodium and fats (Thabet et al. 2010; Dayani *et al.,* 2012).

Many bacterial, fungal and other pathogens have been well studied on date palm; fungal pathogens are considered as one of the most serious pathogens and cause a significant reduction in date palm growth, development and production (El-Hassani *et al.,* 2007; Abass *et al.,* 2013).

Different species of the genus Nigrospora have been isolated and identified as a true endophytic pathogen on numerous plants. For examples, The species of N. oryzae (Berk and Broome) Petch is hosted by rice (Rice grain spot disease) and maize (Maize root rot) (Mew and Gonzales, 2002; Saunders and Kohn, 2008). Whereas, N. sphaerica (Sacc.) Mason has been isolated from decayed banana fruits (Esposito *et al.,* 1962) and spotted leaves of blueberry plants (Wright *et al.,* 2008).

Both of these two Nigrospora species were reported to infect and cause disease in date palm. Abass et al. (2006) were able to isolate and identify the species of N. oryzae from heavily infected date palm leaves with leaf spot disease in 2006, and in 2011-2012 they reported the species of N. sphaerica as a true pathogen of date palm trees which exhibited severe symptoms of leaf and stem spot diseases (Abass *et al.,* 2013).

The present study aimed at the separation of these two species of Nigrospora genus according to their morphological, molecular and pathological levels on different cultivars of date palm.

MATERIAL AND METHODS

1-Fungal isolates

N. oryzae and N. sphaerica were isolated from heavily infected date palm leaves with spot symptoms, most leaves were collected from cvs. Al-Sayer and Hillawi. The isolation was conducted on a Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol at 25 ° C according to Abass et al. (2013). Briefly, heavily infected leaves of date palm were brought to the laboratory and sectioned into small pieces of 1-2 cm2, and sterilized with sodium hypochlorite (10% of commercial chlorox), subsequently rinsed in distilled water and placed on PDA plates.

2-Morphological identification of N. oryzae and N. sphaerica

The hyphae and conidia were examined in 7-d old colonies grown on PDA plates. The morphological identification was performed according to Matsushima (1975). Specimens were examined using a Zeiss AxioLab compound optic light microscope (AxioLab.A1, Fisher Scientific, Germany). Micrometric data was based on measurement of 100 individual spores, hyphae and conidiogenous cells.

3-Extraction and purification of fungal DNA

The procedures used for fungal genomic DNA extraction, purification and ethanol precipitation were according to Zolan and Pukkila (1986). Briefly, a single-spore cultures were placed on Potato Carrot Agar (PCA) medium at 25 °C for 7 days. The mycelium and conidia were collected (approximately 10 g) and ground with liquid nitrogen at room temperature, then extracted with 600 µL extraction buffer [1% hexadecyltrimethylammonium bromide, 0.7 M NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% 2- mercaptoethanol], vortexed and incubated at 60 ° C for 30 min. An equal volume of chloroform:isomyl alcohol (24:1, v/v) was added, tubes were then centrifuged 5 min at 13000 rpm. The aqueous phases were recovered into fresh tubes containing isopropanol and followed by a second centrifugation for 1 min. The DNA pellets were resuspended in 300 µL of TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].

4-Primers description and PCR amplification

Universal primers (ITS1 and ITS4) were selected for molecular identification of Nigrospora species. The sequences of primers were: ITS1: 5': TCCGTAGGTGAACCTGCGG-3', which hybridizes at the end of 18S rDNA and ITS4: 5': TCCTCCGCTTATTGATATGC-3', which hybridizes at the beginning of 28S rDNA (White *et al.,* 1990). The Polymerase Chain Reaction (PCR) was carried out in 0.2-mL polypropylene tubes with a total mixture of 50 µL consisting of a 4 ng of gDNA template, 5 µL of 10× polymerase buffer, 8 µL of dNTPs (1.25 mM), 1 µL of Taq DNA polymerase (Roche) and 1 µl of each primer, and distilled waster up to 50 µL.

The thermal cycler used was equipped with a heated lid (M. J. Research Inc., Waltham, Massachusetts, USA). The PCR cycle was set up as follow: 5 min initial denaturation and enzyme activation at 95°C, followed by amplification for 35 cycles at 95°C for 1 min, 55° C for 1 min and 72° C for 1 min with a final extension at 72° C for 10 min (Rodrigues *et al.,* 2011).

The PCR products were resolved by horizontal electrophoresis in a 2% agarose gel after staining with ethidium bromide (approximately 0.2-0.5 µg/mL). The PCR products were sequenced and analyzed by comparison with all available sequences in the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) using the Basic Alignment Sequence Tool (BLAST): (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

5-Susceptibility of different date palm cultivars to the infection with Nigrospora species

Five date palm cultivars (Al-Sayer, Hillawi, Zahdi, Leloy and Kantar) were chosen (because of their heavily infection symptoms of leaf spot) to determine the level of their susceptibility to the artificial infection with N. oryzae and N. sphaerica under the laboratory conditions. following the procedures of Abass et al. (2013) using mycelium plug inoculation on detached healthy date palm leaves. Briefly, five pieces of leaves (approximately 1.5 cm in length) per cultivar were surface-sterilized and rinsed in sterile distilled water five times. A wound of 0.5 cm diameter and 0.5 cm depth was made by a sterilized cork borer, and a 0.5 cm mycelial plug from N. oryzae and N. sphaerica colony grown on PDA was placed inside the wound and sealed with parafilm. A sterile PDA plug (0.5 cm) served as a negative control was used. The inoculated wounded leaves were placed in 200 mL flasks containing 20 mL sterilised distilled water and kept at 25°C for 30 days. The development of symptoms was monitored and the diameters of resulting necrotic lesions around the wound were measured according to Bachillor and Ilage (1998). The reisolation of the pathogen from the inoculated leaves, to fulfil Koch's postulates, was conducted on PDA plates as described above. The current test was repeated twice to confirm the results, the average of these experiments were considered for analysis

6-Extracellular enzyme analysis

The most important enzymes of both Nigrospora spp. were assayed as below:

6-1- Cellulase activity

N. oryzae and N. sphaerica were grown on YEPA (0.1 g yeast extract, 0.5 g peptone, 16 g agar in 1 litre of distilled water) supplemented with 0.5% (w/v) N-carboxymethyl cellulose. Each plate was incubated at 25 ° C. The plates (9 cm diameter) were flooded with 5 mL of Congo red (0.1%) and then destained with sodium chloride (1%) for 15 min. The clear zones around the colonies were measured by taking the average of three directions on each Petri dish.

6-2- Protease activity

The protease activity of N. oryzae and N. sphaerica was assayed following the procedures described by Amirrita et al. (2012) on GYPA medium (1 g glucose, 0.1 g yeast extract, 0.5 g peptone, 16 g agar in 1 litre of distilled water) amended with gelatine (0.4% w/v). Both GYPA and gelatine were sterilized separately by autoclaving for 20 min. Saturated aqueous of ammonium sulphate was used (5 mL/ plate) to flood the cultures. The saturation of ammonium sulphate was done by dissolving a 75 g of ammonium sulphate in 100 mL of distilled water. The clear halo around the colonies indicating the proteolyitc activity and was measured by taking the average of three directions on each Petri dish.

6-3-Lipase activity

The procedure of Sierra (1957) was followed to determine the lipase activity of N. oryzae and N. sphaerica. Briefly, the medium of Peptone Agar Medium (PAM) (10 g peptone, 5 g NaCl, 16 g agar in 1 litre of distilled water) supplemented with sterilized Tween 20 at 1% (v/v) was inoculated with fungal colony plugs of 0.5 cm of tested species and incubated at 25 ° C. The clear halo indicating the lipase activity.

RESULTS

1-Morphological and molecular characterisation of Nigrospora species

Both N. oryzae and N. sphaerica were isolated from heavily infected date palm leaves with spot symptoms, most leaves were collected from cvs. Al-Sayer and Hillawi (Fig. 1) .After 7 days of culture on PDA plates, both species of Nigrospora grew rapidly and produced white colonies, initially, and then became brown to dark brown due to the abundance of sporulation (Fig. 2 A and B).

The species of N. oryzae produced a single-cell conidium of 14 -16 µM in diameter; each conidium was born on hyaline vesicle at the tip of the conidiophore of 4.5-6.0 µM. The conidium shape was ranging from spherical to black subspherical with the hyphae diameter at 7 -9 µM (Fig. 2 C).

The species of N. sphaerica, a single-cell conidium was produced at the attenuate apex of conidiophores which were 7-9 µM in diameter, spherical to oblate, solitary, black with smooth-walled and about 19 -20 µM as a diameter. The diameters of hyphae were 8 -11 µM (Fig. 2 D).

The results of molecular characterization of Nigrospora species emphasizing on the Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA) with ITS1 and ITS4 primers showed that the ITS sequence analysis had a 99% of identity with a total of ~515 bp for N. oryzae, and ~500 bp for N. sphaerica (Fig. 3).

On the basis of morphological characterization and molecular analysis of ITS region, the identity of Nigrospora species was revealed to be as N. oryzae and N. sphaerica.

2-Susceptibility test of five date palm cultivars to the infection with Nigrospora species

The results of susceptibility test of five different date palm cultivars which were Al-Sayer, Hillawi, Zahdi, Leloy and Kantar, proved the ability of both tested species of Nigrospora to induce spot symptoms on all tested cultivars

after artificial inoculation at laboratory. Generally, N. oryzae was more aggressive species on all detached healthy leaves of date palm cultivars compared to N. sphaerica (Table 1). The overall average of lesion diameter was 1.83 cm in leaf treated with N. oryzae. The symptoms of leaf spot developed as an oval to spherical shape with a green blackish centre. Al-Sayer cultivar was the most susceptible among tested cultivars to the artificial infection with both Nigrospora species where the lesion diameter was 2.50 cm. In contrast cv. Leloy showed the lowest level of susceptibility showing 1.1 cm-lesions after 30 days of inoculation; whereas, all tested cultivars in negative control remained symptomless during the incubation period up to 30 days post-inoculation (Fig. 4). N. oryzae and N. sphaerica were consistently recovered from lesion tissues and reidentified fulfilling Koch's postulates.

3-Extracellular enzymatic activity of N. oryzae and N. sphaerica

The two species of Nigrospora spp. were screened for the activity of their extracellular enzyme, including cellulase, protease and lipase. Both N. oryzae and N. sphaerica showed positive results for cellulase and protease enzyme assay, while no indication for any activity with lipase assay in Nigrospora species (Table 2, Fig. 5). It's noteworthy that N. oryzae was the most active in the cellulase and protease analysis compared to of N. sphaerica.

DISCUSSION

Date palm is considered as one of the most ancient cultivated palm trees in the world providing fruit (dates) as a food source for thousands of years (Sulieman *et al.,* 2012). In Iraq, date palm cultivation encounters several constraints among which the wide spread of fungal diseases presenting a serious threat for growth and development of date palm (Abass *et al.,* 2006). Several important fungal pathogens have been isolated and identified as a causal agent of damaging diseases, including leaf spot disease (Alternaria, Graphiola, Pestalotia, Microsphaerella and Phoma), inflorescence rot (Mauginiella scattae), neck bending (Ceratocystis paradoxa), root rot and fruit rot (*Aspergillus*, Alternaria, Fusarium and *Penicillium*) (Al-Juboory, 2005; Abass *et al.,* 2006; Al-Sheikh, 2009). Most of these diseases have been concentrated in the date palm orchards nearest to the river banks, such as Shaat-Al-Arab River in Basra province where the high level of humidity could contribute to the spread of these fungal infections (Abass *et al.,* 2013).

Regarding the disease of leaf spot, several fungal genera have been isolated and identified as a true pathogen on date palm in Iraq, including: Alternaria, Pestalotio, Mycosphaerella, Phoma and Nigrospora (Abass *et al.,* 2006, 2013). Two different species have been found to be a leaf spot pathogen which belongs to the genus of Nigrospora. N. oryzae and N. sphaerica (Abass *et al.,* 2006, 2013). In the present study, both species of Nigrospora were successfully grown in vitro and exhibiting rapid proliferation on PDA plates at 25° C. However, the morphological examination showed that the sizes of conidia and conidiophores as well as the hyphae diameter could be a reliable parameter for discriminating between these two species. Most importantly, the conidia diameter which were larger in response to N. sphaerica (up to 20 µM) compared to N. oryzae (up to 16 µM). The molecular identification with ITS primers (ITS1/4) revealed the identity of both pathogenic species of Nigrospora. The sequence data alongside with BLAST search proved the identity (99%) to be N. oryzae and N. sphaerica thus confirmed the morphological identification. The Internal transcribed Spacer (ITS) regions of ribosomal DNA (rDNA) has a great importance in confirmation of fungal identification; both ITS primers ITS1 and ITS4 were used to amplify these regions which compass the 5.8S coding sequence situated between large and small units (White *et al.,* 1990). The ITS sequencing method has been implied widely for discrimination between many closely related species belong to the genera of Alternaria, *Aspergillus* and *Penicillium* (Henry *et al.,* 2000; Konstantinova *et al.,* 2002; Pashley *et al.,* 2012; Abass, 2013b).

The susceptible test showed that the species of N. oryzae was the most aggressive on all tested date palm cultivars, compared to N. sphaerica. The most susceptible reactions were observed with Al-Sayer and Zahdi cultivars, in contrast with Leloy cultivar which showed the lowest level of susceptibility for both species of Nigrospora. The high level of pathogenicity in the artificial inoculation with N. oryzae on date palm detached leaves could be attributed to the enzymatic and toxic activity of the pathogen, which might be higher in the in the species of N. oryzae compared to N. spaherica. Several toxins have been isolated and identified from the culture filtrate of Nigrospora, such as lactones, most importantly; phomalactone which induced water-soaked lesion of tested leaves (Fukushima *et al.,* 1998).

The degradative enzymes produced by plant fungal pathogens are crucial factors in the pathogenesis involving several biological functions such as host specificity, deterioration of the present study shows positive results of cellulase and protease activity in the culture media. Both N. oryzae and N. sphaerica produced cellulase and protease enzymes but the highest activity was observed in the cultures of N. oryzae. This variation could be attributed to the level of virulence of N. oryzae which was more aggressive on all tested date palm cultivars in contrast with N. sphaerica. It was reported that the host specificity as well as fungal virulence could be one of the explanations of the variations in the enzymatic activity of different plant fungal pathogens such as Mauginiella scattae, Fusarium moniliforme, F. graminearum and F. semitectum (Abass, 2005; Ahmad *et al.,* 2006).

No detection of any lipase activity in both species of Nigrospora when Tween 20 was used as a substrate for lipase enzyme assay. Numerous published paper showed the suitability of Tween 20 as an appropriate substrate for lipase assay in solid medium (Tan *et al.,* 2004; Amirita *et al.,* 2012). The negative result of lipase was reported in different plant fungal pathogens such as Thialoviopsis paradoxa (Abass, 2005).

CONCLUSIONS

Our results indicated that the morphological characteristics, based on the diameter of conidia of Nigrospora spp. are reliable features for fungal identification on the species level. The morphological characterisation was confirmed by ITS sequences and proved the identity of N. oryzae and N. sphaerica. The susceptibility test of different date palm cultivars revealed higher levels of virulence of N. oryzae compared to N. sphaerica.

The variation of enzymatic activity of cellulase and protease between the two species of Nigrospora may suggest an explanation for the significant differences in their pathogenicity on date palm detached leaves. The high level of virulence of N. oryzae could be correlated with the high enzymatic activity. Further investigations focusing on toxicological and histological aspects will help to better understand the nature of pathogenicity of Nigrospora species.

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Figures

Fig. 1. Leaf spot disease symptoms on A and B Al-Sayer cv. C and D Hillawi cv.

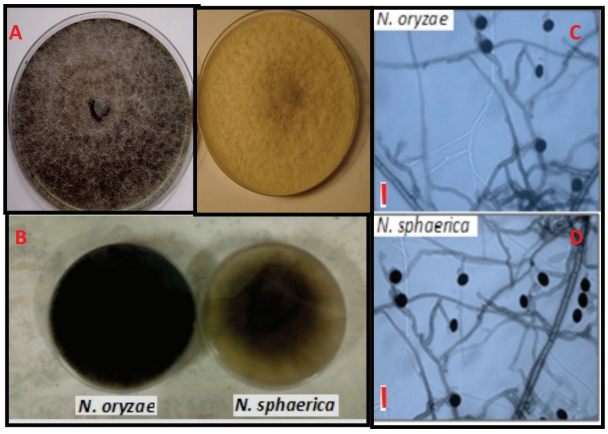

Fig.2 A. 7 days growing culture of N. oryzae and N. sphaerica on PDA plate. B. Reverse growth of N. oryzae and N. sphaerica on PDA plate. C. Microscopic features of N. oryzae . D. Microscopic features N. sphaerica. Bar 20 µm.

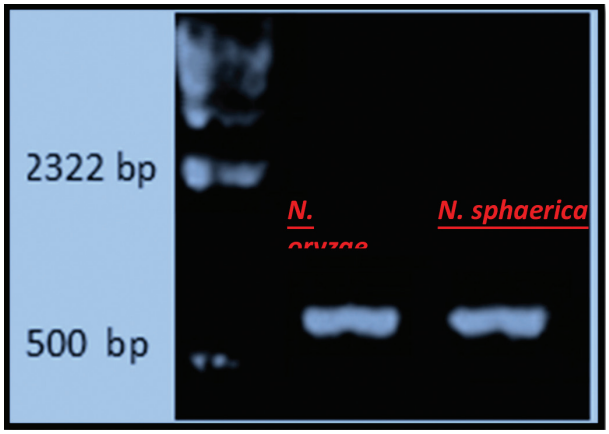

Fig. 3. PCR products of DNA from N. oryzae and N. sphaerica with ITS primers. Lane 1, Lambda HindIII DNA marker; lane 2, N. oryzae (515 bp); lane 3, N. sphaerica (500 bp). The sizes of both fragments were estimated by comparison with lambda HindIII DNA marker (Gene Ruler) and the computer program of Photocapt MW software 10.0, Vilber Lourmat.

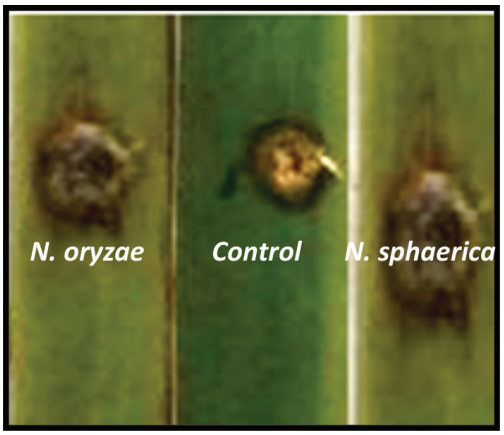

Fig. 4. Infection procedure of N. oryzae and N. sphaerica on date palm detached leaves.

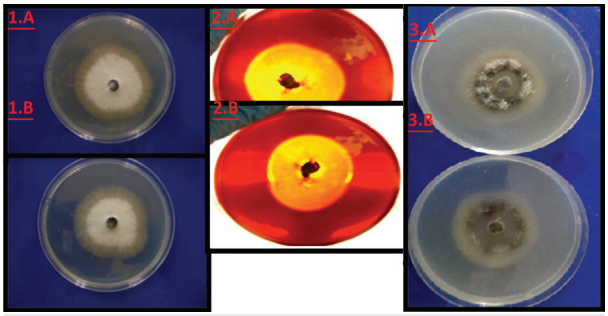

Fig. 5. Results of different enzymatic activity of N. oryzae and N. sphaerica.

1. Protease activity: A. N. oryzae, B. N. sphaerica.

2- Cellulase activity : A. N. oryzae, B. N. sphaerica.

3- Lipase activity: A. N. oryzae, B. N. sphaerica.

Tables:

Table 1. Lesion diameter of different date palm cultivars (cm) caused by two species of Nigrospora

Date Palm cultivar	Fungal species		Average of cultivar
	N. oryzae	N. sphaerica	
Al-Sayer	2.90	2.10	2.50a
Hillawi	1.60	1.25	1.40c
Kantar	1.30	1.50	1.40c
Leloy	1.20	1.00	1.10d
Zahdi	2.15	1.25	1.70b
Average of fungal species	1.80a*	1.42b	

Means within each column followed by the same letter are not significantly different at the $P < 0.01$ level as determined by Duncan's multiple range test.

Table 2. Extracellular enzyme assay of N. oryzae and N. sphaerica.

Nigrospora species	Cellulase activity (mm)			Protease activity (mm)			Lipase activity (mm)		
	R.G.	Z.D.	E.A.	R.G.	Z.D.	E.A.	R.G.	Z.D.	E.A.
N. oryzae	50.0	20.0	+	53.0	15.0	+	45.0	-	-
N. sphaerica	45.0	10.0	+	45.0	10.0	+	35.0	-	-

R.G.: Radial Growth, Z.D.: Zone Diameter, E.A.: Enzyme Activity. + Active; - Inactive.

Morphological characterization of Saudi Arabian date palm cultivars based on vegetative and reproductive traits

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ABSTRACT

The Kingdom of Saudi Arabia is among the top three date producing countries of the world, producing over a million tons of dates annually from an estimated 23 million date palms grown in over 172,000ha accounting for 17% of the global production. The Kingdom has a wide genetic pool of over 400 date palm cultivars.

We studied 11 representative cultivars from eastern, central and western region of the Kingdom. The major vegetative traits studied pertained to fronds, including the leaflets and thorns (spines) on the fronds. With regard to the reproductive traits the number of bunches, bunch stalk, strands, flowers and fruits (fructification) were studied.

Results revealed that the cultivars from the western region had an intensive vegetative growth, as reflected by the higher number of fronds in the cultivars, Ajwah, Anbara and Safawi. This parameter could serve as an indicator to distinguish between cultivars from the date growing regions of the Kingdom. With regards to the length of the fronds, cultivars from the central region viz. Nabutsaif, and Sulaj registered the maximum frond length. Further, the highest number of thorns on the fronds was recorded in the cultivarSafawi from the western region, while the cultivarKhunaizi from the eastern region had longest thorns. It is pertinent to mention that the cultivars from the central region were characterized by few thorns on the frond spaced closely and consequently had more area on the frond for leaflets which could be a genetic character to adapt to the environment. Observations on the reproductive traits revealed that the cultivars from the eastern region recorded the best results with regard to bunch number (Sheshi),length of bunch stalk (Khalas),width of bunch stalk (Reziz) and number of strands per bunch (Khalas). Further, Anbara from the western region recorded the maximum average fruit length and width while the cultivars Khalas and Reziz recorded the maximum length and width of the seed, respectively. Further, the cultivars Sheshi and Ajwa recorded the maximum weight of fruit and seed, respectively. With regard to the form of fruits Khalas, Sheshi and Sokai were oval, Reziz, Ajwah and Nabutsaif were aspheric while fruits of Anbara and Sulaj were semi-cylinderical in form. Seeds of the cultivars studied had three distinct forms viz. semi-cylinderical in Khalas and Ajwah, fusiform in Sheshi, Anbara and Sulaj while it was oval in Reziz and Nabutsaif. These studies form the basis to categorize date palm varieties of the Kingdom into clusters based on the above traits.

Key Words: Date palm, cultivars, Saudi Arabia, morphological characterization

INTRODUCTION

Date palm *Phoenix dactylifera* L. is an important fruit crop of the arid regions of the world especially in the Middle East and North Africa where it has been cultivated since ancient times and is closely associated with the life and culture of the people in these regions. It is estimated that there are 100 million date palms of which 60 % exist in the Arab world. It is believed to have been cultivated as early as 4000 B.C. and has its origin in Mesopotamia (Wrigley, 1995).During the past three centuries, dates have also been introduced to new production areas in Australia, the Indian sub continent, Mexico, southern Africa, South America, and the United States. Dates are a main income source and staple food for local populations in many countries in which they are cultivated, and have played significant roles in the economy, society, and environment of those countries (Chao and Krueger, 2007). Date palm has wide genetic diversity due to a high degree of out breeding (Popenoe, 1992). Zaid and De Wet, 2002 reported the occurrence of3,000 cultivars around the world.

The Kingdom of Saudi Arabia is among the top three date producing countries of the world, producing over a million tones of dates annually from an estimated 23 million date palms grown in over 172,000ha accounting for 17% of the global production. Over 400 date palm cultivars have been reported from Saudi Arabia (FAOstat 2010; Anonymous, 2006). As in all date palm growing countries of the world in Saudi Arabia too, date palm cultivars are region specific characterized by unique vegetative and reproductive traits. These traits play an important role in characterization of a particular cultivar in respect to its adaptation to a particular agro-ecosystem besides impacting the yield and commercial norms of dates. Studies on characterization of date palm cultivars are rare (Baker *et al.,* 1999).

This study pertains to the morphological characterization of major Saudi Arabian date palm cultivars from the eastern, westeran and central regions of the Kingdom based on vegetative and reproductive traits.

MATERIALS AND METHODS

Studies were carried out during 2013 to characterize major Saudi Arabian date palm cultivars from the eastern (4), western (3) and central (4) date palm growing regions of the Kingdom based on the vegetative and reproductive traits. The cultivars studied are presented in table 1.

Studies on the vegetative traits were carried out with respect to the fronds where in observations on several characters viz.number of fronds / palm, length of fronds (m), number of leaflets / frond, length of frond mid-rib with leaflets (m), number of thorns (spines) /frond, length of thorn / frond (m), length of frond mid-rib with thorns (m) and length of frond mid-rib between last leaflet and first thorn (m) were recorded. As regards the reproductive traits fruit bunch characters viz. number of bunches / palm, bunch stalk length (m), bunch stalk width (m) and number of strands / bunch were studied. Further, observations on the physical traits (length, width and forms) of fruits and seeds were also recorded in Khalas, Sheshi, Reziz, Ajwah, Anbara, Sulaj, Nabutsaif and Sugai.

With regard to the frond and bunch characters three replications (palms) per cultivar was maintained. Individual observations were recorded on one frond or bunch in each of the three replicate palms. As regards the fruit characters observations were recorded in three palms per cultivar wherein 15 fruits per palm were maintained. Data on the above characters was compiled and subjected to statistical analysis (ANOVA, p=0.05). Results of the study are presented and discussed below.

RESULTS AND DISCUSSION

Results presented below indicate significant variation among cultivars for all the traits studied.

Among the several vegetative traits studied results presented in table 2 reveal that the date palm cultivars from the western region of the Kingdom had the highest average number of fronds / palm (65.77) with the cultivars Ajwah (66.30) and Anbara (66.00) having the maximum and statistically similar number of mean number of fronds. The least number of fronds/ palm (39.33) were seen from the cultivars in the central region of the Kingdom with the cultivar Sukari recording the lowest mean number of fronds (32.00). Further table 2 reveals that length of the fronds and leaflets / frond were inversely related to the number of fronds/ palm with cultivars from the east recording a least values for mean frond length of 3.70 m and leaflets/ frond of 162.25 as compared to cultivars from the central region which registered the highest frond length of 4.70 m and leaflets/ frond of 205.43. A similar trend was observed with respect to length of frond mid-rib with leaflets, where cultivars from the central region recorded the highest mean value (3.72m), with cultivars from the east registering the lowest mean value for this character (2.57m). It can be inferred that the lower number of fronds per palm for cultivars from the central region of the Kingdom was compensated by higher frond length and leaflets / frond there by sustaining photosynthetic levels in relation to date palm cultivars from the west of the Kingdom where the cultivars recorded higher number of fronds / palm. Though, cultivar wise significantly different values were recorded for number of leaflets on the right and left of the frond, this character was cultivar specific with the same

number of leaflets being recorded for each cultivar on the right and left of the frond mid-rib.Microsatellites analysis of 26 Tunisian date palm cultivars using stable vegetative features showed high polymorphism among the cultivars studied (Hamza*et al.,*2011a).In date palm yield levels are known to be correlated the number of fronds. Nixon, 1957 reported that an average of 7.5 leaves/bunch ratio in the Deglet Noor cultivar was needed to obtainhigh yields of fruit of good quality and also to assure the production of an adequatenumber of bunches the following year. Bacha and Shaheen, 1986 concluded that increasingleaf/bunch ratio up to 9: 1 resulted in increasing yield and improving fruit quality in both Nabutsaif and Reziz cultivars.

Results pertaining to characteristics of thorns (spines) on date palm fronds in major Saudi Arabian cultivars (Table 3) show significant differences among the cultivars studied with Safawi from the west of the Kingdom recording the highest number of thorns / frond (39.00). In general cultivars from the east recorded least mean number of thorns / frond (22.33) as compared to cultivars from the central region of the Kingdom which recorded the highest mean value (26.60).This character was inversely related to length of the thorns on the frond with cultivars from the east recording higher mean values (0.18m) as compared to date palm cultivars from the central region of the Kingdom (0.10m).Variation in this trait (spines) could be a physiological adaptation to different environmental conditions prevailing in the three regions of the Kingdom.

With regard to the fruit bunch characteristics (Table 4), significant differences were recorded for the traits studied with the highest mean number of bunches / palm being recordedin date palm cultivars from the east (12.59) followed by cultivars from the west (11.33) and the central region of the Kingdom (9.23), respectively. The cultivar Sheshi from the east registered the highest number of fruit bunches/ palm (16.33). As regards the number of strands / bunch the cultivars from the east registered the highest mean values (76.75), while the cultivars from the west of the Kingdom registered the lowest mean value (49.33). For this trait, the cultivar Khalas recorded the highest value of 94.30 strands/ bunch.The cultivars, Khalas and Reziz from the east also recorded maximum length and width of bunch stalk, respectively. Yield levels in date palm are known to be influenced by vegetative traits especially the leaf/ bunch ratio (Nixon, 1957; Bacha and Shaheen, 1986). Our findings with respect to the cultivars Reziz and Khalas are in agreement with these reports.

Further from figure 1 it is evident that the cultivar Anbara from the western region recorded the maximum average fruit length and width while the cultivars Khalas and Reziz recorded the maximum length and width of the seed, respectively. Further, the cultivars Sheshi and Ajwa recorded the maximum weight of fruit and seed, respectively (Figure 2). Reports from Saudi Arabia indicate that analysis of the morphological data of fruits revealed a high level of diversity in length-width ratio, colour, shape of the fruit, fruit-base and in the percentage of area covered by the fruit cap. Correlation of morphologic characters with genomic similarity using RAPD markers showed that the fruit shape is one of the characteristics most influenced by genetic variation (Al-Khalifa *et al.,*2012). Studies carried out on the quality norms of premier date palm cultivars from the eastern region of Saudi Arabia (Al-Abdoulhadi, 2011), showed that Khalas recorded the maximum fruit length in all the three categories of large, medium and small sized fruits . With regard to the breath of fruits, the cultivar Sheshi registered the highest values. Further, Sheshi recorded the highest fruit weight values, which in turn influenced the number of fruits per unit weight, with Sheshi recording the least number of fruits per 500g .Sakr*et al.,*2010 from Egypt reported fruit length to significantly differ among the fruits of eight date palm cultivars studied with the cultivar Kuboshy registering the maximum fruit length, while the cultivar Samany registered the maximum fruit width.

With regard to the form of fruits Khalas, Sheshi and Sokai were oval, Reziz, Ajwah and Nabutsaif were aspheric while fruits of Anbara and Sulaj were semi-cylinderical in form. Seeds of the cultivars studied had three distinct forms viz. semi-cylinderical in Khalas and Ajwah, fusiform in Sheshi, Anbara and Sulaj while it was oval in Reziz and Nabutsaif (Table 5).Our results are in agreement with reports by Al-Khalifa et al 2012 for fruit shape of the cultivars Khalas and Sukari. Studies carried out in Tunisia on date palm cultivars to study the morphological and genetic diversity showed significant differences among subpopulations for all traits measured with morphological variation being correlated to fruit maturity period (Hamza *et al.,* 2011b).

It can be concluded that there exists wide variability among the date palm cultivars studied and further molecular analysis will help to determine the relationship among these cultivars forming the basis to categorize date palm varieties in Saudi Arabia into clusters based on the above traits.

Acknowledgement

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Tables

Table 1. Major date palm cultivars from the eastern, western and central date palm growing regions of Saudi Arabia selected for the study

Sr. No.	Date palm cultivars of Saudi Arabia studied		
	Eastern region	Western region	Central region
1	Khalas	Ajwah	Sulaj
2	Sheshi	Anbara	Sukari
3	Reziz	Safawi	Nabutsaif
4	Kheneizi		Sugai

Table 2. Characteristics of date palm fronds in major Saudi Arabian cultivars

Sr. No.	Cultivar	Mean values					
		Number of fronds / palm	Length of fronds (m)	Number of leaflets / frond	Number of leaflets on right of frond mid-rib	Number of leaflets on left of frond mid-rib	Length of frond mid-rib with leaflets (m)
I. Cultivars from the Eastern Region							
1	Khalas	55.00ab	3.87b	175.70c	88.70c	88.70c	2.77c
2	Sheshi	46.70bc	3.43c	145.00d	73.00d	73.00d	2.69c
3	Reziz	56.70ab	3.45c	152.30d	76.00d	76.00d	2.20d
4	Kheneizi	45.30bc	3.98b	176.00c	87.70c	87.70c	2.61cd
II. Cultivars from the Western Region							
5	Ajwah	66.30a	4.05b	199.00b	99.00b	99.00b	3.16b
6	Anbara	66.00a	3.51c	169.30c	87.00c	87.00c	2.64c
7	Safawi	65.00a	4.79a	170.30c	83.30cd	83.30cd	3.40b
III. Cultivars from the Central Region							
8	Sulaj	53.30ab	4.97a	224.00a	113.70a	113.70a	4.08a
9	Sukari	32.00d	4.28b	197.70b	98.30b	98.30b	3.25b
10	Nabutsaif	32.70d	5.13a	186.70bc	92.70c	92.70c	3.97a
11	Sugai	-	4.43b	193.30b	96.70b	96.70b	3.58ab
Regional mean values							
	East	50.93	3.70	162.25	81.35	81.35	2.57
	West	65.77	4.12	179.53	89.77	89.77	3.07
	Centre	39.33	4.70	205.43	100.35	100.35	3.72

Figures with same letters within the column are not significantly different (p=0.05)

Table 3. Characteristics of thorns (spines) on date palm fronds in major Saudi Arabian cultivars

Sr .No.	Cultivar	Mean values			
		Number of thorns /frond	Length of thorn / frond (m)	Length of frond mid-rib with thorns (m)	Length of frond mid-rib between last leaflet and first thorn (m)
I. Cultivars from the Eastern Region					
1	Khalas	27.33c	0.16b	0.87b	0.24c
2	Sheshi	20.33d	0.12bc	0.77b	0.29b
3	Reziz	21.00d	0.13b	0.87b	0.21c
4	Kheneizi	25.67c	0.24a	0.90b	0.23c
II. Cultivars from the Western Region					
5	Ajwah	19.00d	0.12bc	0.75b	0.38a

Sr .No.	Cultivar	Mean values			
		Number of thorns /frond	Length of thorn / frond (m)	Length of frond mid-rib with thorns (m)	Length of frond mid-rib between last leaflet and first thorn (m)
6	Anbara	18.67d	0.09c	0.72bc	0.40a
7	Safawi	39.00a	0.12bc	0.36b	0.34b
III. Cultivars from the Central Region					
8	Sulaj	25.00c	0.08c	0.84a	0.22c
9	Sukari	31.67b	0.08c	0.84b	0.31b
10	Nabutsaif	26.33c	0.14b	0.92b	0.39a
11	Sugai	23.33c	0.09c	0.73bc	0.35ab
Regional mean values					
	East	22.33	0.18	0.85	0.24
	West	25.56	0.11	0.61	0.37
	Centre	26.60	0.10	0.83	0.32

Figures with same letters within the column are not significantly different (p=0.05)

Table 4. Characteristics of date palm bunches in major Saudi Arabian cultivars

Sr. No.	Cultivar	Mean Values			
		Number of bunches / palm	Bunch stalk length (m)	Bunch stalk width (m)	Number of strands / bunch
	Cultivars from the Eastern Region				
1	Khalas	12.33b	1.67b	0.04b	94.3a
2	Sheshi	16.33a	0.98bc	0.05b	79.3b
3	Reziz	11.00b	1.06b	0.14a	63.7b
4	Kheneizi	10.67bc	1.24ab	0.04b	69.7b
Cultivars from the Western Region					
5	Ajwah	9.00c	0.81c	0.04b	65.0b
6	Anbara	12.00b	0.79c	0.04b	37.3a
7	Safawi	13.00b	1.05b	0.05b	45.7c
Cultivars from the Central Region					
8	Sulaj	12.33b	1.24ab	0.04b	74.7b
9	Sukari	6.67d	0.88c	0.04b	54.7c
10	Nabutsaif	8.67c	1.48a	0.04b	74.7b
	Sugai	-	-	-	-
Regional mean values					

Sr. No.	Cultivar	Mean Values			
		Number of bunches / palm	Bunch stalk length (m)	Bunch stalk width (m)	Number of strands / bunch
	East	12.59	1.11	0.07	76.75
	West	11.33	0.88	0.06	49.33
	Centre	9.23	1.20	0.04	68.03

Figures with same letters within the column are not significantly different (p=0.05

Table 5. Forms of date fruits and seeds in major Saudi Arabian cultivars

Sr. No.	Cultivar	Fruit form	Seed form
1	Khalas	Oval	Semi-cylindrical
2	Sheshi	Oval	Fusiform
3	Reziz	Aspheric	Oval
4	Ajwah	Aspheric	Semi-cylindrical
5	Anbara	Semi-cylindrical	Fusiform
6	Sulaj	Semi-cylindrical	Fusiform
7	Nabutsaif	Aspheric	Oval
8	Sugai	Oval	Fusiform

Figures

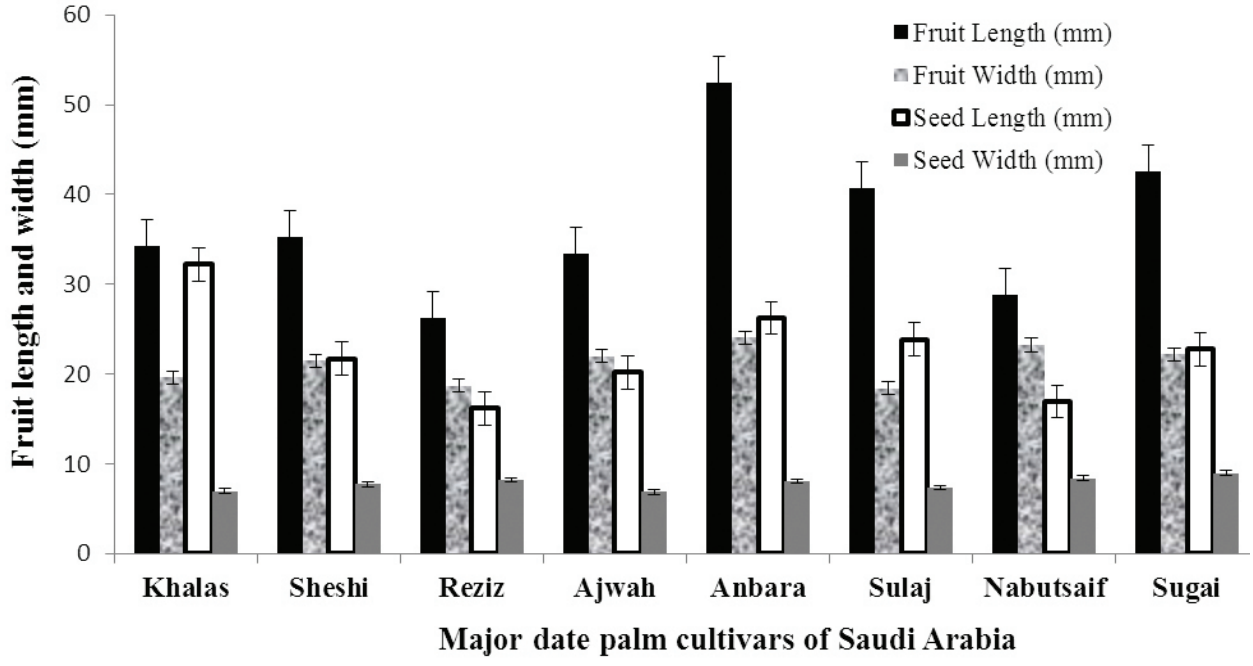

Figure 1. Physical traits (length and width) of date fruits and seeds in major Saudi Arabian date palm cultivars

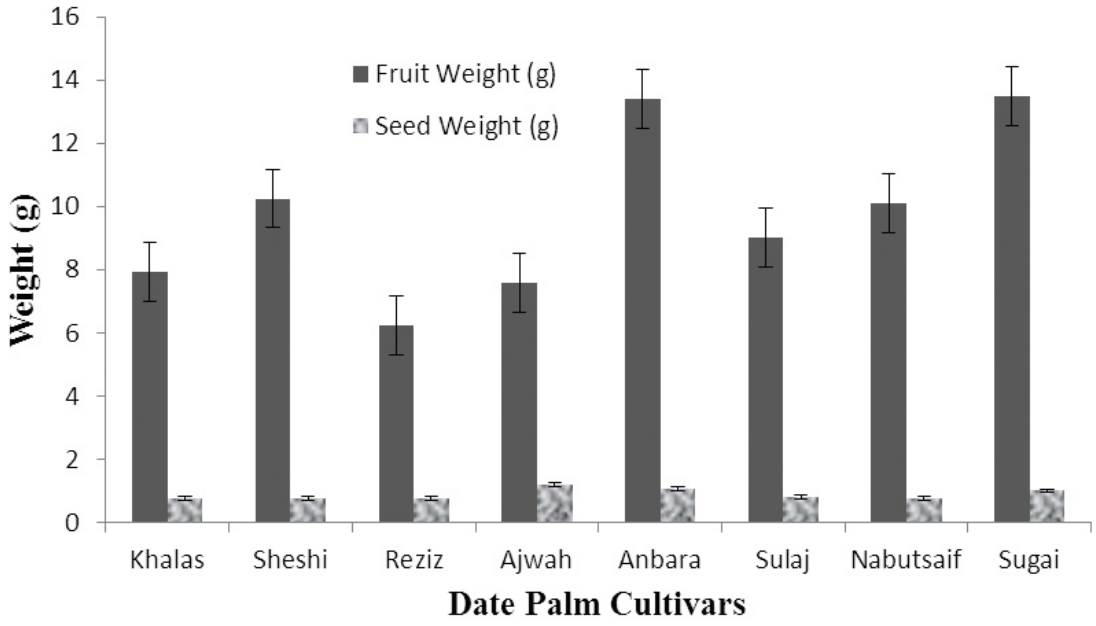

Figure 2. Weight of date fruits and seeds in major Saudi Arabian date palm cultivars

The calendar of date palm care in Abu Dhabi Emirate

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ABSTRACT

This paper highlights the most important care priorities of Date Palm (DP) tree as one of important loops that aimed enhancing the quality of the performance and develop maintenance operations for greening projects in Abu Dhabi (AD), in line with the social responsibility of the municipality and embodies its vision to ensure the better quality of life and sustainable environment for AD residents. At the beginning, it reminds the importance of this blessed tree, which mentioned in the Holy Koran and the Sunnah. It touches the latest monitoring of DP varieties in UAE, and their groups according to the date maturity, explains and deals with clarifying local scheduling operations for DP care. The paper shows the importance of periodic emphasis on correct applications care according to their scheduled time that leads to stronger growth and better production which are the important components of mechanical and biological control against various pathogens or insects that can affect the DP tree at all stages, the paper presents the most mistaken practices in the performance of DP care and utilize them positively on behalf of the development of DP care. At the end, the paper shows most of the DP services throughout the year in calendar table. To replace the notion "that there is a pesticide for each disease or insect by concept "that each disease has its causes that must be limited", therefore the calendar warns about the importance of periodic emphasis on correct applications care according to their scheduled time. As a conclusion, it can be adopted as a guide for developing the DP care practices in Abu Dhabi Emirate.

Keywords: local scheduling operations, Rachis Base, Bunch Curving, pollination, fruits thinning technique,, fruit bunching. DP Sanitary Care.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), which includes more than 1,500 varieties, is one of the Arecaceae family that includes 225 genera and around 2600 species. It's one of the oldest fruit trees, said it may sprouted at least more than ten thousand years ago. DP cultivated in ancient Mesopotamia, Sumer and Assyria, as well as in ancient Egypt (between the Nile and the Euphrates). Its wild origin is unknown, but the fixed belief and excavations signs confirmed that it's cultivation in the east of the Arabian Peninsula, including UAE, back to 4000 years BC, from which spreads to other parts of the world . Some sources indicated that the palm family is the oldest among the flowering plant families, as confirmed by some of the discovered fossils to being back to nearly 120 million years.

Due to the high viability of this species to withstand harsh environmental conditions, it has spread to extendible areas of the Islamic and Arabic world, where extending from North Africa (Morocco), to Egypt up to latitude 17 north to the south, also extends to latitude 15 north in Sudan to fall thereafter to the latitude 10 to the north and along the Red Sea and the Aden Gulf, including the northern parts of Somalia and it's south border extends within Asia to include the south shore of the Arabian Peninsula up to Pakistan, where the palm belt extends to the north up to latitude 32 north in Iraq and Iran. It is also widely grown in the tropics and subtropics of the both parts of our planet.

In United Arab Emirates it is considered one of the most important commercial fruit species, as well as it enjoys under the exceptional promise because of its social importance and heritage prestige.

Due to this stature and unique beauty its cultivation have spread across the country for many purposes

on public and private farms and parks, protective windbreaks and forest shelterbelts around cities and along internal and external roads.

The latest monitoring to inventory DP varieties in UAE revealed the existence of more than 225 varieties of pistilate ones: about 70 varieties from seed origins (Jish) focused in Ras Al Khaimah Emirate. It has also been monitoring unlimited number of Male (Fahal) varieties from which studied nearly 20 variety.

For more clarification, the varieties have been divided into: too early (as Naghal), early (Halawy, Sayre, Gura, Heri), moderate early (Prem, Khenaizy, Khadrawy, Thwaira Nmici), In the mid-season (Boumaan, Baglat Mtawah, Boscri, Khalas, Derry, Zamili, Shbibi, Sage, Soufri, Madjool, Nabhtat Saif, Maktoum, Red Hilali, Shishi, and Aljishosh: as Alawan,Ramli, Estooh,Soeah, Alaq and Tabaq), moderate delay (Barhi, Khashrm, Khesab Liwa, Sulhtana, Rziz, Lulu, Deglet Nour, Shakhul) and late (Algebri, Jish Makran, Red Farhd –Liwa, Yellow Farhd –Al Ain, Naghal Hilali and Saudi Hilali) and too late (Khesab, Hilali, and Um Al Fanajeen).

The variation in the maturity periods of date's varieties, stretching from late May to late October, has a great importance in organization of harvest works and on controls the operations of dates marketing.

To take the advantage of this positive feature in reducing the size of the works, it is vital important to conduct scheduling operations for fruit harvest according to their proper association with variety and followed health conditions. This helps to minimize the extent of the damage and losses that can cause fruits due to the attack of many insects. It also considering that the professional commitment, in charge of the performance level of all operations and services for the palm care, is the basic substrate relying upon its development and giving longevity.

OPERATIONS AND SERVICES FOR THE DATE PALM CARE

Irrigation Control

Is one of supporting essentials for the DP growth, its productivity and safety if its quantities adopted properly. That's where the disadvantages of water excess (preferred by the red weevil): the spread of fungal diseases, nutrient deficiency, level rise of the ground water and delayed growth and fruit ripening... and others. The disadvantages of water deficit (preferred by borers): leading to weakness, slow growth, flowering delay, small and low quality fruits and get phenomenon of alternate fruit bearing... and others.

So take into account the attention of appropriate amounts of irrigation during the formative stages of pollen and fruiting as they have a vital influence on the amount of the crop, and are reduced during the winter season and the start of fruits coloring depending on the stages of maturity of the crop until the end of harvesting.

Generally, there are two irrigation periods determined by the outcome of the general and local environmental conditions and the norms related to the quality and quantity of water, location, adopted irrigation method, palm age and its variety, soil properties and level of its preparation, the intensity and novelty of cultivation and the operations of care and maintenance, namely:

1) Summery (04/01 to 10/31): the amount of irrigation at a rate of one cycle every 3-5 days.

2) Wintry (11/1 to 3/31): the amounts of irrigation at a rate of one cycle every 6 -7 days, which is reduced by at least 25%.

The omission of any factor would upset the accurate calculation of DP water requirements exposing them to the problem of inadequate suitability that contribute to the deterioration of DP status and declining their growth and productivity. It is also necessary to act on the maintenance of irrigation systems before each period (semiannual) to ensure the supply of required irrigation amounts.

Palm, Basins & Site Cleanliness

DP with all its parts requires regular monitoring of cleanliness through the stages of fruit development. It's especially after the end of Beesir stage and while entering into Tamar stage, as well as through fruits ripening and harvesting, which increases the chances of their fall on all DP parts and around the basins and of its beauty distortion. All of which will form safe food haven for many insects (red palm weevil, DP borers, Dubas, spiders, scales).

The presence of these insects on the different parts of DP trees leads to infection .It is therefore recommended to collect all larvae, pupae, nymphs, complete insects to eliminate them and prevent their reproducing.

It takes into account the periodic care of basins and site cleanliness by the immediate removal of all vegetative, organic green and dried litters caused by the different DP service operations as well as the fallen fruits. It also requires to get rid of any infected or sick DP and deteriorating plants within the site by applying the proper means, which would constitute hotbeds for the outbreaks of disease or pests. Note that the proper implementation of this process would enhance the degree of benefit from all of these residues in many industries.

This application will ensure the maintenance of a healthy environment free from any harmful pathogens that would prejudice the safety of DP growth and its development.

Digging, Weeding& Root Covering

Includes the surface hoeing of the soil, weeds and alien plants removal that growing on the DP basins periodically, by extraction from their roots and collected with other organic waste for preparing an organic fertilizer. Attention to soil hoeing could provide the appropriate medium within the DP basin by improving soil ventilation, moisture, improve its texture, avoiding any competition effect on nutrients and removal the safe reproductive hot beds of insects.

Taken into account in the case of revealed roots or their appearance, at the base the DP trunk, working on aggregating the soil on and around it. This process is a very important within the basin area (especially in terms of DP plantations) to encourage the production of offshoots around their mothers in the early stages).

As always taken into account after the completion of this process to install the irrigation bubblers to ensure the uniformity of water distribution in the whole basin. It is advisable to use the organic mulch materials for the purpose of reducing water consumption.

Fertilization

The fertilization program should be based on the results of samples analyzes of soil, water and plant tissues that derived scientifically to represent the target site or farm.

1. **Organic Fertilization (OF):** The sandy soil properties highlight the importance of mixing the decomposed treated organic fertilizer with it. The DP needs a rate not less than 5 kg / yr of its age, and with proportionate amount according to the age and variety (over 10 years by rate not more than 50 kg / Palm).

We can start adding OF at the end of October-December through circular spreading in the DP basin then mixed with surface soil up to depth, which does not affect the root system. The goal is to encourage the DP growth and strengthen its immunity against diseases and resistance to pests and configured to good production.

2. **Chemical Fertilization(CF):** The adding of CF in addition to the OF, in particular the compound one enriched with trace elements, will work to increase the productivity of palm significantly compared with non- fertilized. This has its effect on improving the fruit quality in terms of weight, size, and the fruit flesh. They can be added during the same period for the organic fertilizer by:

- 150 g / year of offshoot's age for ages younger than 10 years.
- 1.5 kg compound fertilizer / mature palm for ages greater than 10 years.

This means that the total amount of fertilizers for trees greater than 10 years is:

- 50 kg / palm organic fertilizer + 1.5 kg / palm compound fertilizer with trace elements

Notes:

- The DP generally needs: 200 g nitrogen +75 g phosphorus +100 g potassium / year of age (high nitrogen fertilizer).
- When require to support the growth of female spathes we can add during January 100 g of urea / year or 1 kg per palm that exceeded 10 years.
- The following element's compounds can be mixed with the compost as it's added :
  1. Superphosphate for phosphorus supplies.
  2. Potassium sulfate for potassium supplies.
  3. Copper sulfate for copper supplies.
- Urea can be mixed with compost only before its usage. It considered avoiding mixing the nitrate compounds or ammonium sulfate with compost.
- Avoid using untreated compost from unknown origin, being one of the sources of infections.

Pruning

It is preferably to conduct and complete the operations of frond removal and rachis base cutting during December - January, when the numbers of weevil insects as little as possible, while avoiding cutting any green frond.

Frond Removal

The process of cutting dry fronds (after 3-7 years of their life) from the bottom of the DP canopy (can be made after Fruit bunching when required), or the damaged or diseased ones.... and any other reasons.

It's done to the level that supports the above green fronds and facilitates the process of climbing and working in the surround heart of DP canopy. It includes the bunch's removal and the old Rachis Bases. The pruning works to liberate the crown, increase its ventilation and exposure to the sun and facilitate working through it to discover any injuries or infections. (Generally the mature and good cared DP tree produces 15-20 fronds annually).

To protect the DP canopy and the bases of the lower green fronds against the climatic fluctuations, it is recommended to leave at least two lines of pruned dry fronds without cutting their rachis bases and consider not cutting any green frond.

Rachis Base Cutting

It done by cutting the outer part of the remaining rachis after the pruning process (1-2 years after pruning) in a sloping cut to the outside. Such a process can spare the palms from the insects attack (red palm weevil and bunch's borer), which prefer to hide and lay their eggs in such places (where dark, safe and appropriate niche thermally).

Note: Rachis base cutting not recommended to conduct for the new DP trees only after 7 years from their cultivation and upon reaching the height of 1.5 –2 m, taking into account not to remove any green fronds from them (except when necessary to facilitate their care or when they touching the soil surface).

Spine's Removal

Usually take place before flowering to facilitate the pollination services; bunch's care and their distribution within the areas surround the heart of DP. During this operation, taking into account the full care to avoid any offences against the fronds.

Offshoot's Removal& Planting

It is always necessary to liberate the DP mother trees from aerobic offshoots that grown on the trunk and the offshoots around it (their ages above 3 years). It is recommended not to leave more than three offshoots around each palm to encourage the growth of other offshoots, facilitate the services of mother palms, minimize the attack by the insects, especially the red weevil (which prefers fleshy offshoots), maintain their health and safety and exclude the nutritive depletion.

Usually the process of offshoots removal from their mothers and their planting may conduct in the spring: March-May or in the autumn: August-October. The preferable season under the local environmental conditions is autumn, where the survival percentage can exceed 80%.

Treatment of Cutting & Wounds Areas

It is necessary, after the pruning or rachis base cutting or offshoots removal or for any broken fronds and bunches, taken into account to close or treat the injured areas to prevent the odor emissions that attract the insects, especially the red palm weevil.

Pollination

Pollination is one of the most important and delicate biological processes that could limited the level of date's quality and productivity. Therefore it is necessary to pay a serious attention from the beginning of the emergence of early mature spathes (end of January - February) to proceed the male palm trees from dry fronds and spines and facilitate their collection before pollen's blowing out. After the confirmation of good analysis of pollen's efficiency, whether stored or new, we have to prepare the pollens for pollination. For the purpose of pollen's preservation, it is preferable to use the paper bags to cover the male spathes while monitoring the female spathes bloom especially for early flowering varieties in order to pollinate them successively, this operation also continue during March.

Pollination process takes place, usually in the morning and within the range of 25-35 °C, during February-March and April and in accordance with the blooming-time of female spathes. This varies according to different varieties and environmental areas. Some are required immediate pollination after the cracking of the female spathes cover (sage and Ashrasi) and the other can be extended from 10-15 days (Lulu, Jish Habash, khistawi). Although stigmas of female flowers remain receptive for several days, it is better to pollinate the inflorescences as soon as cracks open. Most of varieties must pollinate during the 2-4 days and before strand's greening. It is advisable to refrain pollination during rainy or windy weather. After the verification of the quality of male varieties and their spathes ripeness, and providing the entire requirements of pollination we can be setup to carry out the process.

During the manual pollination, we inserted 6-24 strands of male flowers in each inflorescence and covered all directly with punched paper bags (Bagging) to support the fruit hold percentage, increase the quantity and quality of production and reduce the incidence of Lesser Date Moth insect (Humera).

The automated pollination contains the processes of pollens' extraction and their automated delivery to the stigmas of female spathes. The amount of used pollens varies depending on their vitality and varieties, noting that the effect of pollens on the fruit quantity and quality also linked with used male variety.

The pollination is one of the most effort processes in comparison with other DP care services. Therefore, it requires action to reduce these efforts through the use of skilled labor in order to ensure high success rates in shortest time and through the adoption of automated pollination, which has the effective impact on raising the economic returns of DP cultivation.

Fruit thinning

3. **Strand's thinning**

This first stage conducted during February - March and after 2-3 days of cracking of the female spathes (before pollination). It is a favorite stage for varieties that have long strands, and are either:

- To cut off the end of the strands by 25% (7.5 -10 cm) and leave approximately 50-60 strands per each spathes.
- Remove the strands by 30% from the heart of spathes.

The general advisable practices to remove strands from different places of each spathes or cut their ends by a third for long strands varieties, such as: Barhi, Khesab and Deglet Nour or cut a fraction of the strands or do not cut anything, as in short strands varieties, such as: Khalas, Hallaway and Khadrawy. Later it also advised to remove a number of the fruit per each strand as in varieties: Naghal, Barhi, and Madjool.

4. bunch's thinning

This second stage conducted, during the period from mid-March until mid-May depending on DP varieties and after insuring the completion of pollination, by thinning the bunches and maintaining 6-8 bunches per each mature healthy palm that has 9-12 green fronds per each bunch and this rate will vary according to variety, age and service.

In order to minimize the load of the DPs and for investment purpose, it's advisable to direct their energy consumption towards improving the fruit volume and quality by taken into account to remove the following bunches during this phase:

- Bunches with small and weak fruits load and which close to DP heart.
- Non-pollinated and poor-pollinated bunches.
- Infected and late emerged bunches.
- Bunches emerging between the old fronds sites (weaken growth)

The process of bunch's thinning requires into account the balance of bunches' distribution (load distribution) to prevent the possibility of any impact on palm curve, especially for fast-growing varieties such as: Barhi and Lulu.

To estimate the size of this process we have to consider the impacts of many variations; DP age, variety and growth status that affected by general and local environmental conditions.

This has been proven that the highest yield and the best fruit qualities can be obtained when the thinning is 25% of the bunch's number and 10% from the length and the number of strands in the bunch.

Bunch Curving and Support

It is conducted by bending the bunches via pulling them among the fronds located on the perimeter of the DP canopy, making a regular and balanced distribution around it to facilitate care services, harvesting and confirming its safety .

It is carried out on the early stages of fruit ripening - during the two phases of Kimri and Khalal (Beesir) from the mid or the end-April to mid-July (April-July), so after a month to a month and a half on the process of pollination, depending on the variety and particularly before wooding or hardening the stalks of long bunches.

The bunch supports complementary and enhanced bending process, where it conducted by linking the bunches (prefer fibrous cords) from their stalks with the upper fronds or carry them (whichever is the best to support the bunch safety in the right position.

The advantages of this process are; to examine and observing the bunches health and fruits, make sure they are free from any injuries (especially Lesser Date Moth) and to avoid any breakage that may happened as with non- supported bunches.

Fruit Bagging

This process conducted, at the beginning of fruits discoloration while entering into Beesir stage, by covering bunches by net bags in order to avoid fruits fall, facilitate their collection and maintain the cleanliness of all parts of DP trees and the basin areas, as well as protect them from birds and reduce the chances of being attacked by insects

DP Sanitary Care

It is necessary to perform a periodic monitoring for the health status of palm plantations with adoption a calendar for integrated control operations: bio-mechanical as well as protective, when needed to use organic pesticides. As it is not advisable in any case to use chemical pesticides in the cases of minor injuries, note that the usual spraying of pesticides has no significant effect on these pests, especially when the infection inside the Palm.

To rehabilitate the neglected farms should be adhered to implement the following acts:

- Get rid of all dead, diseased and weak growth trees and all dead organic waste according to the approved rules.
- Emphasis on sterilization of wounds after pruning the affected vegetative parts and spraying appropriate fungicides according to the approved conditions. With a constant concern for sterilization machines and tools used in pruning especially during the transition from sick to healthy tree.
- Implement all the operations of DP care according to the approved schedule calendar. Making sure to remove the fiber and all the dead waste and tissue from the hidden media of rachis bases also it is advisable to choose a good treated organic fertilizer.
- Work to ensure the regularity of palm spacing depending on the variety and site requirements to facilitate mechanization and maintenance services.

- Monitor the quality of water sources for irrigation and their suitability in terms of freedom of any neither pathogen nor Insect cause.

Notes:

- Emphasize exceptional choice of strong male varieties donating good fertility and compatible with planted varieties because of their impact on the quantity and quality of the yield and harvest period.
- On DP farms, the intercropping may contribute in minimizing the palm attraction to insects on condition that the soil has prepared well and the planting spaces of DP are adequately for grown varieties.
- Need to adhere strictly to the timing and the terms of correct control. Commonly, it is conducting randomly after symptom onset, the pests have disappeared and achieved their damages, and may be laid eggs (most resistant stage) that enhances in repeating the pest cycle.

Yield's harvest

The yield of date palm trees passes through the following stages of growth and ripening:

- Hababok: 4-5weeks after pollination of female flowers (February- mid of March).
- Kimri stage: 5 - 6weeks after the former, it considered the longest stages of growth (mid of March -end of April).
- Beesir or Khalal stage: 3 - 4 weeks after Kimri (to end of May), like: Barhi, Khesab, khenaizy, Lulu, Hilali, Samani, Zaghlool, Hayany and Khalas.
- Rutab stage: 3 - 4weeks after Khalal (June - the beginning of August), like: Naghal, Manaz and the varieties of high-value marketing like: Deglet Nour, Madjool, Segae, Sukkari, Anbara and many others that can be maturated industrially.
- Tamor stage (full maturity): as in most varieties, like: Dayri, Halawy, Khadrawy, Thoori, Zahidi, Sayer and Aliig.

Therefore, on the months of May-June start preparing for yield's harvest operations and once the very early varieties entering the Rutab stage to prevent the loss of their marketing value. And, following these operations at the end of July for the moderate early varieties and for the most varieties in August - September where the preferred harvest at the beginning of Tamor stage. In order to maintain the quantity and quality of the dates yield and at the lowest loss level requires an attention to the following basics:

- The dates harvesting must conducted according to the priorities of maturity periods of varieties and consumer desire for the particular variety in order not to lose their market value.
- Preparation of all supplies, equipment and machinery for this process, collection, sorting and drying of the crop before marketing fundamentalist.
- Noted, if operations of harvesting, collecting and drying are not set up well according to the proper rules, the crop would be subjected to damage in quantity and quality.

Negative Consequences Of Negligence The Scheduling Of DP Care

The absence of schedule of DP care will work on providing suitable hidden media or environments to stimulate the spread of many pathogens and insect. For example: neglect hoeing operations and good soil preparation would provide a middle course of many Borers proliferation within the DP basin, and the use of bad compost can be a major source for the spread of Bunch Borer.

It is worth noting, that the vulnerable and neglected palm farms provide suitable environments for exposure to many insect injuries, such as: Red Palm Weevil, Dubas, Lesser Date Moth, Date Spider Mite and many Date Palm Beetles, in addition to the risk of many fungal diseases, such as: Black Scorch Disease and Belaat Disease.

In addition to that these farms provide more encouraging and possibility of exposure to some physiological diseases (functional) through the interference of their degraded conditions with the effects of the local environment factors, for example: head curvature (top) and apex abnormality and fruits wilting and falling.

Some mistaken and common practices

- Negative professionalism of laborer (adapted to unintentional negative practices) that contributes to decline the performance of care services.
- Improper care services, like: cutting the green fronds, left what intrudes and inhibits the DP growth and not caring to do all the services according to their norms.
- Pollinate the small new cultivated offshoots as well as the aerial offshoots that growing on and around their mothers.
- Wrong pollination that linked with low performance level, incorrect timing process and bad quality and incomplete maturity of the male spathes.
- Left the weaken bunches without thinning or those with very little fruit load and which have unbalanced scatter or bunches grown from the DP heart.
- Left bunches numbers that not commensurate with DP vegetative system (the total number of health green fronds).
- Bad investigation of the health status of palm plantation.

- Weak hiring of security and public safety controls during the maintenance operations.

Basic measures in regularity of DP care services

- Secure of skilled manpower (foremen and workers), proportional to the size of DP care services (DP numbers and their varieties and age).
- Apply professional practices for all DP services according to their annual calendar taking into account the effects of variety and location.
- Provide all the supplies, equipment and techniques for facilitating the perfect performance of all DP services, proportional to the size of manpower.

Recommendations

- Periodical verification to give the priority to add treated organic fertilizer to support soil fertility and improve its mechanical properties for the drainage and moisture retention, with permanent thought to invent or introduce any technologies that have economic feasibility.
- Apply preventive and protective controls through strict implementation of agricultural quarantine controls, as well as non-trading of any offshoots or infected palms (especially from and to nurseries or between different palm plantations) for the purposes of planting or marketing.
- Serious thinking to create adopted windbreaks designs to be established around the DP plantations, due to their significant impact in reducing the evapo-transpiration inside the protected farms that reflected positively on DP productivity.
- For the purpose of improving DP growth and production, it needs to adopt a study project to evaluate all DP marketing varieties and according to their environmental distribution across the emirates.
- To avoid the DP farms from many problems, researchers should work to identify cultivation zones of DP varieties according to the appropriate outcome of the environmental conditions for each definite variety.

The interest in such measures and others would contribute to cover the costs of water irrigation and various care services and can achieve a good return from the exports of various DP products which flows into support of food security and the national economy.

As a general rule:

Replace the notion says: “for each disease or pest there is a pesticide”, by adopting the practical concept “that each disease or injury has its causes that must be treated and limit their impact.”

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The Annual Calendar of Date Palm Care in Abu Dhabi Emirate (Al Mashhadani, 2014)

Services	1		2		3		4		5		6		7		8		9		10		11		12	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Irrigation Control																								
Irrigation System Maintenance																								
Palm, Basins & Site Cleanliness																								
Digging, Weeding																								
Organic Fertilization																								
Chemical Fertilization																								
Pruning																								
Old Fronds & Bunches Removal																								
Rachis Base Removal																								
Spine's Removal																								
Offshoots removal and planting																								
Treat wounded areas																								
Collect Mature Male Spathes																								
Pollination & Bagging																								
Fruit thinning																								
Strand's thinning																								
Bunch's Thinning																								
Fruit Bunching																								
Fruit Covering																								
DP Sanitary Care																								
Growth Stages of Palm Fruit																								
Hababok																								
Kimri																								
Bessir (Khalal)																								
Rutab																								
Tamor																								
Fruit Harvesting																								
Pest & Disease Control	Controllable by adoption of this calendar of DP care and by conducting the safe applicable measures of Integrated Pest Control (IPC)																							

Adoption of biointensive IPM to enhance the development of organic date palm cultivation in the Arab countries

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ABSTRACT

Recently, there has been an increased demand on organic date in the developed as well as developing countries including the Arab countries. However, there are certain challenges for developing organic date palm cultivation in the Arab countries which are the main global producers of dates. Since the pest management practices should be applied in accordance with the valid standards of organic date production, the insufficient management of pests is one of the major reasons of such constraints. In fact, knowledge of organic-agriculture standards and available required information of the all components of IPM strategy is very important to develop adequate programs to manage the pest problems in organic farming; available data indicate that the basic components include: biological and ecological aspects of the target pest, field monitoring and scouting, threshold /action levels and natural controls, whereas, the methods and tactics which are commonly used as main and potential components to implement the IPM program include: regulation and legislative interventions, agricultural methods, attractants and pheromone traps and biological control. When the organic management practices alone cannot prevent or control pests, a biological or botanical substance may be applied through biointensive integrated pest management (Bio-IPM) programs. Consequently, bio-IPM is not just about management of pests alone, it is a sustainable crop production system based on sound eco-system analysis. However, there are certain constraints on its wide-range implementation in the Arab region. This paper highlights the current situation of IPM levels in Arab region and the need to overcome constraints and encourage the implementation of bio-IPM programs in organic date palm farms.

Key words: Organic date, Pests, Adoption and implementation, Biointensive IPM

INTRODUCTION

Organic agriculture includes all agricultural practices that promote the environmentally, socially and economically sound production of food. Crop production and pest control methods in organic agriculture are governed by strict standards and rules imposed by the International Federation of Organic Agriculture Movement (IFOAM) and national regulations. These standards applies to the unprocessed and processed products that carry or are intended to carry descriptive labelling referencing organic production methods. On the other hand, organic production is generally associated with different challenges; the major challenges include low quality palm cultivars, poor farm management, pest and disease control (and inadequate IPM: integrated pest management), harvesting, processing and marketing, shortages of national qualified and trained staff and labour, and insufficient research and development (Mahmoudi, *et al.,* 2008). Regarding organic date palm cultivation, dates shall refer to organic production only if they come from a farm system employing management practices that seek to nurture ecosystems in order to achieve sustainable productivity; and that provide weed, pest and disease control through a diverse mix of mutually dependent life forms, recycling of plant and animal residues, crop selection and rotation, water management, tillage and cultivation (United Nations, 2003; Azadi *et al.,* 2006; El-Zemaity, 2007b; Safwat, 2007).

Some Arab producers have diversified into organic production of dates. For example, Tunisia export certified organic dates

to the European countries, the main market is Germany. Tunisia exported 678 tones of organic dates (The official production Figure was 107 000 tones for all varieties) in 2000-2001, up 60 percent from 425 tones in the previous season (Fruitrop, 2001).The recent data indicated that in 2011, 6,000 tons of organic dates were harvested in Tunisia, of which 4,000 tons (67%) was exported; 68% of this went to Germany, 11% to the United States and 7% to Morocco (Source: Freshplaza.com). Although Tunisia accounts for only 2 percent of world date production, its share of global exports in value is 21 percent. It represents 55 percent of EU imports in value. Tunisia exports about the same quantity of processed and natural dates. Algeria came the second with a market share of 20 percent of EU imports in value. The official production Figure in 2000 was 365 000 tones for all varieties. Algeria exports more natural dates than processed dates, as there is a lack of processing capacity. The quasi-totality of Algerian dates is destined for France (Fruitrop, 2001). Among organic fruits, date palms are of major importance in other Arab counties such as Egypt, UAE, Palestine and Saudi Arabia. Organic date production of these countries is locally distributed (Hartmann, *et al.,* 2012).

As conventional date palm the organic date palm cultivation and its fruits could be subject to attacks by several pests that are, in most cases, well adapted to the oasis environment. The main causes of date palm damage include insect pests, rodents and diseases (Naturland, 2002; Blumberg, 2008; Mahmoudi, *et al.,* 2008). The damage caused by such pests is considerable and leads to heavy economic losses. Most of pest control operations employing pesticides are either restricted or not permitted not only in organic date but also at all in organic products. The principles of pest control in organic farming are based on: (i) prevention of infestation, (ii) avoiding the contamination of organic foods by any form of infestation, (iii) avoiding any contamination of organic foods with plant protection products, and (iv) the use of substances which not adversely affect the environment. Generally, IPM is a set of management activities that farmers implement to maintain the intensity of potential pests at levels below which they become pests, without endangering the productivity and profitability of the farming system as a whole, the health of the farm family and its livestock, and the quality of the adjacent and downstream environments. Consequently, IPM is not just about management of pests alone, it is a sustainable crop production based on sound eco-system analysis. However there are certain challenges that constrain its wide range implementation (Guan Soon, 1996; Dhaliwal and Heinrichs, 1998).

Considering all mentioned previously, it is highly expected that the Arab countries which are the main producers of dates will face some of constraints in developing of organic date cultivation. So, the present paper highlights the current situation of IPM levels in Arab region and the need to overcome constraints and encourage the implementation of bio-IPM programs in organic date palm farms.

DISCUSSION

Possible causes of date palm damage in organic cultivation

All parts of offshoots and mature date palm tree could be exposed to the infestation by different abiotic as well as biotic disorders includes pests and diseases. The main pests which include insects; mites; plant pathogens; weeds; rodents and birds are similar in most of the Arab countries (Fig. 1). Some of these pests are considered serious pests in certain countries, whereas considered moderate or minor pests in the others. Among of these pests the two main serious pest-threats in date palm plantations now are the Red Palm Weevil (*Rhyncophorus ferrugineus*) and the fungal disease Bayoud (*Fusarium oxysporium*) (Calcat, 1959; Carpenter and Elmer,1978; Al-Azawi, 1986; Howard *et al.,* 2001; Zaid *et al.,* 2002). Other date palm disorders such as environmental, physiological and propagation factors could be causes of considerable damage in each country. The occurrence of date palm pests and/or injury symptoms is depending on the development-stage and the environmental factors. Naturland, 2002 reported that most of the problems concerning disease and pests have different causes, i.e. (a) monoculture cultivation and use of non-resistant and/or of few varieties; (b) insufficient distance between species that grow to the same height, failure to trim agro forestry systems; (c) unfavorable soil conditions like degenerated or poor soil, soil not deep enough for roots, lack of organic material, high salinity etc and (d) unsuitable site conditions (deep water table, insufficient irrigation, drought, temperature, high rainfall level etc.). It is worth mentioning that the absence of adequate management of such disorders could cause considerable damage and lead to heavy economic losses.

Requirements of pest control in organic date palm

Pest control (including insect pests, diseases and weeds) shall be centered on organic management practices aimed at enhancing crop health and minimizing losses caused by such pests. When the organic management practices alone cannot prevent or control possible pests, a biological or botanical substance or other substances may be applied (British Pest Control Association, 2002). However, the conditions for using the substance shall be documented in the organic plan. Pest management plan should be based on essential considerations: (1) appropriate practices should be adopted to prevent pests and avoiding the contamination of organic food by any form of infestation from microorganisms, insects or other pests; (2) control measures should be achieved

mainly by means of scrupulous cleaning procedures and hygiene controls adopted within and around warehouse and storage areas, food preparation areas and for all contact surfaces, within particular emphasis given to the frequent and regular cleaning of inaccessible areas; (3) the permitted pest control substances which does not adversely affect the environment may be used if these practices are ineffective and must be used without any risk of contamination; and (4) the use of chemical means of pest control should be kept to minimum, and restricted substances should lead to the organic products losing their organic status. These emphasize that the pest management practices should first involve the removal of pest habitat and food; second, the prevention of access and environmental management (light, temperature and atmosphere) to prevent pest intrusion and reproduction; and third, mechanical and physical methods (traps), permitted lures and repellents. On the other hand the operator shall, however, ensure that any pest control substance used does not come in contact with the organic raw materials or product, and shall record the use and disposition of all such substances. These requirements could be implemented through the bio-IPM strategy which emphasizes on proactive measures to redesign the agricultural ecosystem to the disadvantage of a pest and to the advantage of its parasite and predator complex (UIUC, 1997).

Biointensive integrated pest management (Bio-IPM) system and planning the suitable program

Biointensive integrated pest management (bio-IPM) is a system approach to pest management that is based on an understanding of pest ecology. It begins with steps to accurately diagnose the nature and source of pest problem, and then relies on a range of preventive tactics and biological measures to keep pest populations within acceptable limits (Leslie and Cuperus, 1993; Steiner, 1994; Altieri, 1994). Reduced risk pesticides are used if other tactics have not been adequately effective, as a last resort and with care to minimize risks. Generally, bio-IPM has many of the same components as conventional IPM, including monitoring, use of economic thresholds, record keeping, and planning (El-Zemaity, 2006). On the other hand, bio-IPM system is affected by several factors such as: economic costs and benefits of individual components; emergence of new pests, resistance or unusual weather problems; the skill and competence of field personnel conducting scouting, designing tactics and assessing effectiveness of given strategies; the impact or importance of preventive practices; availability, or lack thereof of effective alternative pest management products; and the complexity of interactions among pests, beneficials, cropping practices and control measures. Moreover, all IPM programs, regardless of the situation, share the components of monitoring the pest population and other relevant factors; accurate identification of the pest; determining injury levels and threshold that trigger treatment; timing treatments to the best advantage; spot-treating for the pest; selecting the least – disruptive tactics; evaluating the effectiveness of treatment to fine –tune future actions and educating all people involved with the pest problem (El-Zemaity, 2007a).

Good planning must precede implementation of any IPM program, but is particularly important in a biointensive program. Planning should be done before fruiting season because many pest strategies require steps or inputs, such as beneficial organism habitat management that must be considered well in advance. Attempting to jump-start an IPM program in the beginning or middle of a season generally does not work.

The current situation of IPM levels in the Arab region

Measuring the success and improving the efficiency of IPM actions by adopting better application practices require accurate evaluation of the current management programs. The success of the IPM program can be measured by the ability to maintain infestation levels below threshold level or a given % in a target area. In fact, information on the degree of adoption and evaluation of IPM practices in the Arab countries is very lacking (El-Zemaity, 2006). Regarding the actual implementation of IPM along the Arab region, it could be classified to 3 categories of adoption (low, medium and high – level IPM), with the exception of chemical control level which no practices of IPM (or no IPM) are employing and the system is essentially dependent routinely on insecticides (El-Zemaity, 2013). The adopted practices of the three categories may include: (1) low – level IPM, employing at least the most basic IPM practices-scouting and applications in accordance with economic threshold/ area–wide management; (2) medium – level IPM, some preventive measures, coupled with efforts to cut back on broad spectrum of insecticide use and (3) high – level IPM, integration of multiple preventive practices to control the insect without relying on insecticides such as in organic farming. The high – level IPM is the most advanced IPM and termed as the bio- intensive IPM. The actual percentages of current IPM adoptions levels in each Arab country are not well known. This may require encouraging researches on the evaluation of the adopted IPM programs under the local condition of each country. Such researches have become necessary to improve our understanding of the success and true impacts that can be expected from the commonly used IPM practices.

Successful implementation of Bio-IPM approach

1. Pest identification (pest diagnosis)

A crucial step in any IPM program is to identify the pest. The effectiveness of both proactive and reactive pest management measures depend on correct identification. Misidentification is actually harmful and costs time and money. Help with positive identification of pests may be obtained from university personnel, private consultants, the cooperative extension service, books and websites. After a pest is identified, appropriate and effective management depends on knowing answers to a number of questions related to the pest life cycle and the role of agricultural practices in enhancement its natural control. So, monitoring (field scouting) and economic injury and action levels are used to help answer such questions as well as to make adequate analysis of the overall situation of a date plantation through agro-ecosystem analysis.

2. Agro–ecosystem analysis (AESA)

The objective of AESA is to build awareness of the relationship that exists between organisms in the environment and to make good management decisions. The AESA should be done weekly to monitor conditions of crop, weather, soil, pests (including diseases and weeds) and beneficial organisms (predators and parasites). To conduct proper AESA, it is highly recommended to spend some time discussing the needed information, observations and recording results. This discussion should lead to the correct way to observe date palm plantation and chosen observed trees. In-field observation of represented sample tree should carefully be observed for the presence of any pests, beneficials, injury symptoms and signs on the different tree parts (growing point, inflorescences, leaves, fruits, trunk/stem, off shoots, bulb, roots, whole plant). Soil surface also observed for any ground – dwelling pests or beneficials. The results of observed pests and associated organisms, as well as different leaf spot disease symptoms should be recorded on AESA chart (Fig.1A&B) or presented in inspection table or illustrate AESA chart.

3. Proactive tactics of bio-IPM system

Cultural control and pest- resistant cultivars - All agricultural methods should be utilized to create a non-suitable environment for the multiplication of the pest and offer suitable habitat for beneficial organisms. On the other hand cultivars should be resistant to major pest(s), appropriate for the area, commercially available, should have appropriate mode of resistance and must have a market.

Mechanical and physical controls - Methods included in this category utilize some physical components of the environment, such as temperature, humidity, or light, to suppress the pest. Common examples are covering the fruit bunches with plastic nets, flaming, soil solarization, and plastic mulches to kill weeds or to prevent weed seed germination.

Biological control - Biological control is the use of living organisms - parasites, predators, or pathogens - to maintain pest populations below economically-damaging levels, and may be either natural or applied. The first step in setting up a biointensive IPM program is to assess the populations of beneficials and their interactions within the local ecosystem. This will help to determine the potential role of natural enemies in the managed agricultural ecosystem. It should be noted that some groups of beneficials (e.g.,spiders, ground beetles, bats) may be absent or scarce on some farms because of the lack of habitat. These organisms might make significant contributions to pest management if provided with adequate habitat. Possible natural enemies of the main date palm pests are listed in Table (1).

Reactive pest management options

Since, IPM requires continuous assessment of a situation (UIUC, 1997), there are certain key questions that must be answered before implementing any management strategy such as: Is treatment necessary? What are the alternatives to prohibited substances that can inhibit pests? What are commercial sources for these alternatives? Where should the treatment talk place? When should action be taken? and Which tactics should be used?. The answer of these questions required to emphasize that the mere presence of a pest doesn't necessarily warrant treatment. Some times a fairly large population of pests can be tolerated while other times the presence of a single pest is intolerable. In addition, the determination in treatment will vary among individuals. Also, pest managers must look to the whole system to determine the best place and timing to solve the problem. A successful IPM program is based on taking "a whole system" or eco-system approach to solve a pest problem (Leslie and Cuperus, 1993). We must think of both the living and non-living components when determining which approach to take, and each component has impact on every other component (Altieri, 1994).

Choosing practices/ tactics

Organic control practices for the main pests (i.e. insects, diseases and weeds) are based on non-chemical sanitation, physical, mechanical, cultural, and biological means as well as organically-permitted products including approved chemicals. Since no single practice is effective for all possible pests that threaten the crop, a combination of such practices is necessary. Proactive and reactive practices or tactics should be chosen to achieve the organic control measures (Fig. 2). Steiner, 1994 reported that the proper selection of control

techniques is among the bases of successful management of insect pests. During the growing season there are numbers of practices to maintain healthy plants including adequate fertilizing, irrigation and mulch. Preventive devices, sticky colored yellow, black light and pheromone traps are excellent trapping techniques and can be used as survey tools, and may offer protection to plants. These practices could make fields unattractive to pest species. However, sometimes this may be not enough when the levels of pest populations or damage are not acceptable. The use of bio-pesticides including microbial products, botanicals and biochemical substances in these cases are necessary practice. Permitted and restricted pest management tools in organic farming are listed in Table 2.

CONCLUSION

Adoption and overcome of bio- IPM constrains to improve the effectiveness of current programs used in organic date cultivation is needed. Furthermore, new management programs for organic agriculture need to be designed, where the crop environment discourages pest development. Also, the role of training of organic farmers and farm groups should be emphasized as a key feature of successful programs in learning and implementing new practices. Meanwhile the IPM continuum could be achieved according to the following action plan:

1. Define an appropriate IPM continuum for the country or the region.
2. Establish at what stage we are now.
3. Establish realistic objectives in consultation with all stakeholders.
4. Recommended action to industry and to government.
5. Establish new positions of crop management specialists.
6. Recruit a professional with research and extension expertise in the area of bio - intensive IPM.

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Tables

Table.1. Possible natural enemies of the main date palm pests

English/ Scientific name	Possible Natural Enemis	Source
White scale/ *Parlatoria blanchardii* Targ	Hemisarcoptes malus, Chrysoperla vulgaris, Cardiastethus nazarenus, Coccinellidae (29 species), Nitidulidae (5 species), *Mycetaeidae* (1 species), *Aphytis mytilaspidis*, *Cybocephalus nigriceps, Cybocephalus rufi frones, Chilocorus bipustulatus var. iraniensis* and *Chilocorus sp*.	FAO, 1995.
Red scale/ Phoenicococcus marlatti. cockerell,	General predators, such as *Pharoscymnus anchorago* (Fairmaire), are considered as active predators.	Zaid et al., 2002
Red palm weevil (RPW)/ Rhynchophorus ferrugineus Oliv.	Entomopathogenic nematodes (*Heterorgabditis species or Steinernema sp.*) - Entomopathogenic fungi (*Beauveria bassiana, Metarhizium anisopliae*) - Entomopathogenic bacterium (*Bacillus thuringiensis*)	Dembilio and Jacas, 2013.
The dubas bug/ *Ommatissus binotatus var. Lybicus* (De Bergevin)	The egg parasitoid *Pseudoligosita babylonica* (*Hymenoptera: Trichogrammatidae*).	Hassan et al., 2003; Hubaishan& Bagwaigo, 2010.

Table.2. Permitted and restricted pest management tools in organic farming.

Permitted	
- Carbon dioxide, nitrogen, freezing, heating and vacuum treatment. - Mechanical, sound or light barriers. - Electric flying insect control units. - Tamper resistant bait stations. - Pheromone traps & sticky boards. - Diatomaceous earth & amorphous silica. - Particle film barriers (processed kaolin clay). - Sugar esters - Compost teas.	- Botanical products. - Microbial products. - Organically approved chemicals (Bordeaux mixture, sulfur and copper) Restricted (Substances used only in case of immediate threat to organic foods becoming unfit for consumption due to infestation) - Pyrethrum derived only from a natural source. - Synthetic pyrethroids for the treatment of sealed units.

Figures

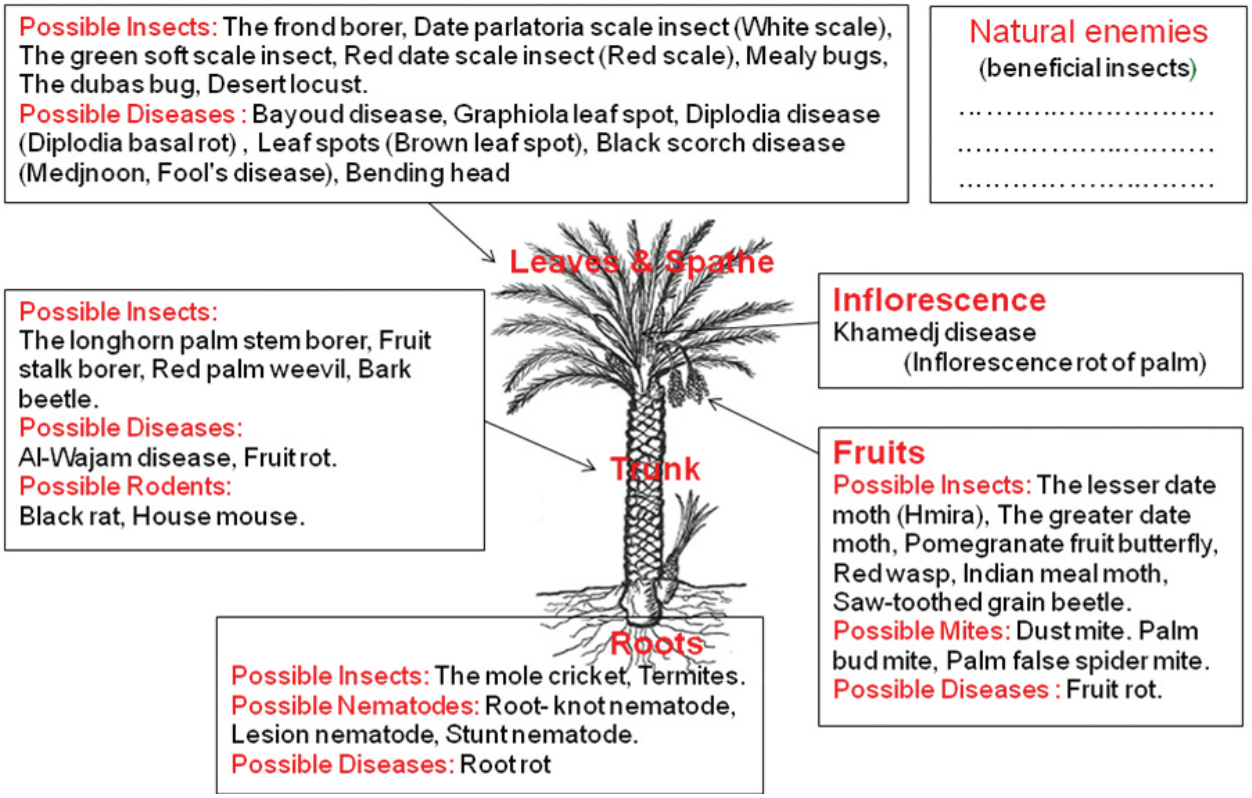

Fig.1A. Agro-ecosystem Analysis Chart for Date Palm

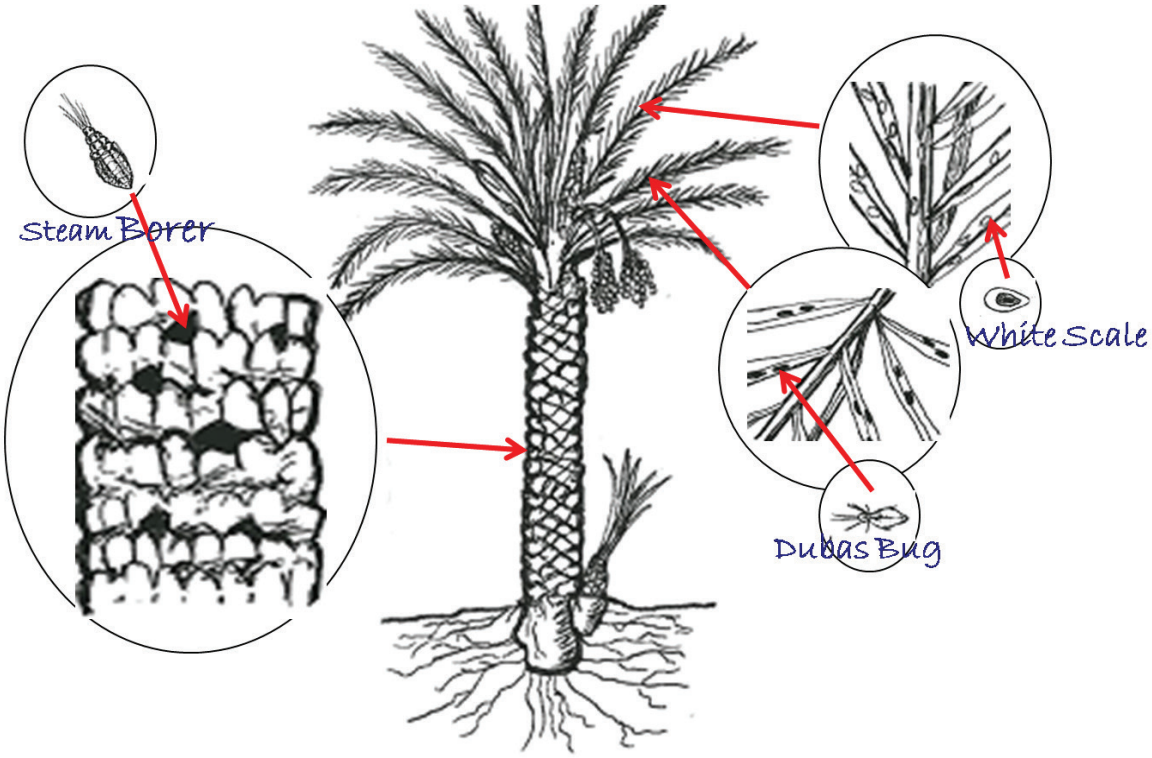

Fig. 1B. Agro-ecosystem Analysis Chart for Date Palm

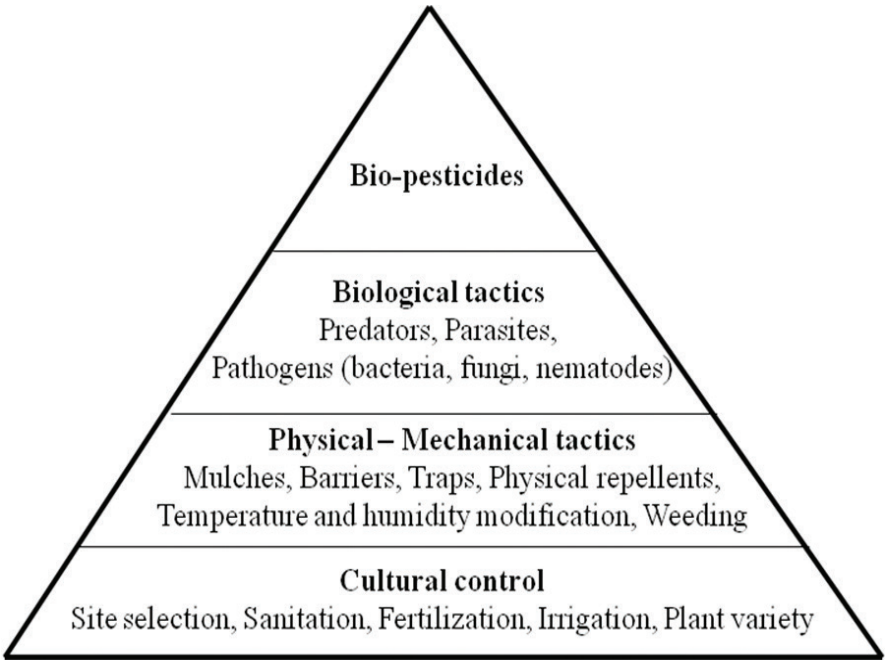

Fig.2. Pyramid of bio-IPM tactics (proactive and reactive options).

Relationship between Iranian male and female date palm elite cultivars by codominant marker (microsatellite)

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ABSTRACT

High and excellent Date production is related to pollen use from elite male stocks, which is called Metaxenia phenomena. Using 8 pairs of SSR primer investigated genetic diversity and relationship between 7 male and 19 female Date palm cultivars collected from throughout Iran. DNA extracted via Sambrock CTABbr2 procedure (1998) with little modification. All the primers could produce bands. Collectively 56 alleles produced with average 7 alleles per locus. No more than 2 alleles per locus found that means Date palm is diploid plant. Mean excepted heterozygosity was 0.723±0.045, mean observed heterozygosity 0.753±0.029 and polymorphism information content (PIC) 0.670±0.049. Inbreeding coefficient (F) mean was -0.346±0.219 showed these cultivars did not obtain by near crossing, and need wide crosses to new elite female or male Date palm cultivar improvement. Based on Nei's distance indices and Ward clustering obtained dendogram separated the studied cultivars into 3 groups that follow the geographical distribution. Distance between males and females were not more than distance within males or females. Therefore could not select proper male stock for specific female cultivars based on their distance. But observed heterozygosity was high for elite male stokes, so can conclude that may select it on its higher heterosigosity. Heterosis of male stock beside establishment of high pollen production potential, increased general combining ability between pollen mass and ovules of spots because its more diverse produced pollens, result to improve fruit quantity and quality that result to Xenia and Metaxenia.

Key words: Date palm, SSR, Iran, Male and Female Cultivars, GCA, Genetic Distance, Xenia and Metaxenia

INTRODUCTION

Date palm tree is perennial monocot, heterozygous, and belongs to the Arecaceae family and is one of the most important horticultural crops in arid and semi-arid countries. Researchers have proved that the type of pollen used for pollination is effective on quantitative and qualitative characteristics of fruits (seeds and edible parts) (1). Most scholars believe that Date palm originated from Mesopotamia (Middle East) or Africa. Therefore, Iranian male stocks probably have lot of diversity. Hamwieh et al (2010) considered distribution palm as function of environmental factors and diverse ecosystems in arid climates throughout many countries. To date many studies had taken on diversity of Date palm cultivars in the world; also, some investigations have been made to determine best male pollinator for famous female cultivars. Billotte et al. (2004) designed 16 primers using Billotte et al. (1999) protocol and were able to show palm polymorphisms across the genome. Mirbabaee et al. (2011) have used a slightly modified Fiasco method to design new 9 primers for SSR positions of Date palm. Arabnezhad et al (2012) using the new AAG and AG-rich

repeats SSR markers and adaptation them with the cloned DNA sequence of Date palm were able to design 25 primer pairs, and studied genetic diversity among 16 genotypes from different geographical areas, but only 22 primers were able to demonstrate polymorphism between cultivars. Akkak et al. (2009) identified 41 binary rich repetitive sequence microsatellites from palm gene library, and after screening 17 microsatellite primers, studied 31 cultivars of collected palm trees from California and Algeria. Al-Ruqaishi et al (2007) were used microsatellite markers for screening and analysis of genetic diversity in Date palm genotypes derived from somatic embryos in Oman. Ahmed et al. (2009) were used microsatellite markers for analysis of genetic diversity and relationships among 15 varieties of female palm trees of Qatar. From 16 primers, that have ability to raise transparent bands, 10 primers demonstrated more clearly single bands, but six other primers did not show clear bands. However Hamwieh et al (2010) stated although the typical features such as codominance and high polymorphism, microsatellite markers are less used in Date palm trees.

The first step to improve of Date palm is determination of suitable male for pollination the popular cultivars. Therefore, some efforts have been made around the world, including Iran (Tallaie A. R., Panahi B. 1997; MirShekari A., Hassan-Pour A. 2001; Jahan-Tigh A., Panahi B. 2009). However, these researches even fail to cover important commercial cultivars of key Date producer areas. In addition to hardness of working in the key Date production areas, because too apparent similarity between palm cultivars is difficult to distinguish them based on morphological properties. According to classical breeding theory, the distance between the male and female may results in more production due to more consistent heterosis, that proposed a hypothesis to determine the proper males. But, this hypothesis is not checked base on genetic relationship between known compatible male and female yet. In other hand, a proper male must to have characteristics such as earliness and high pollen production. Also between molecular methods only codominant ones that are able to identify the exact nature of diversity and relationships between different genotypes. In this order, SSR markers were used in this study to investigate the relationship between Iranian male pollinator and popular Date palm female cultivars beside their variation.

MATERIALS AND METHODS

Plant materiel: In this study, seven known pollinator male and 19 cultivars of commercial females has been collected from the biggest Dates producer provinces of the country, including Khuzestan, Kerman and Sistan and Baluchestan (Fig 1).

Total genomic DNA extraction: The young leaves (which are white or yellow) washed with sterile water to remove the wax. About 200-300 mg of leaves, washed and chopped fine grinding in a mortar with liquid nitrogen, then DNA was extracted follow Sambroke et al. (2004) protocol with some modification. The extracted DNA was dissolved in sterile water and stored at refrigerator temperature for 18 hours. Quantity and quality of DNA were determined using a Nanodrop spectrophotometer and 1% Agarose gel.

PCR and SSR amplification: Amplification reaction was done by Bio-Rad thermal cycler in a volume of 25 µL containing 80-60 ng genomic DNA, 2.5 ml 10xPCR buffer, 0.7 ng MgCl2, 1 mM dNTP, 1 unit Taq enzyme and 1 nM primer. Thermal cycles composed a cycle initial denature 95˚C, 35 round include 30 seconds 95˚C denature, 30 seconds annealing temperature for each primers (Table 1), 45 seconds 72˚C extension temperature, and finally one cycle of 10 min at 72°C extension temperature. Then produced segments separated on polyacrilamid gel 0.8%.

Data analysis: After determining the bands length, data scored based on the presence (1) or absence (0) and saved in Excel. The distance matrix computed on Nie genetic similarity coefficient, diversity indices (Table 2) calculated by GenAlEx6.2 software and phylogenetic tree plotting by SAS based on Ward procedure.

RESULTS AND DISCUSSION

All eight pairs showed high levels of polymorphism with total 57 alleles. Billotte et al (2004) with 16 primers estimated polymorphism rate of about 76% and the number of alleles at each locus around 14. Akkak et al (2009) with 17 primers on 31 cultivars obtained average number of alleles 6.4 per locus, and observed polymorphism 63%. Arbnejad et al (2012) with 22 primers in 16 cultivars estimated 106 alleles overall, with an average of 4.8 alleles per locus, and polymorphism 67%.

Based on the Nie similarity coefficient, no resemblance was seen between Astaamran and Halavy, or Zahdi and Rabbi, which all four cultivars belong to the Khouzestan province and adjacent areas. Most similarity (0.671) was between Ashgar from Khouzestan and Golgoly belong to Sistan and Baluchistan. Cluster analysis (Figure 2) divided the clones into 3 groups. Male Verdi, Astaamran, male Samesmavy, male Ghannami, male Jarvis, Barhi and Zahedi were in first group; most cultivars such as male Sabzparak, Almehtery, male Jalogh1, Halili, male Fenouch, Ashgar, Golgoly, Hemravy, Shkar, Majoul, Mazafati and Rabbi in second group; and cultivars like Barim, Jouzi, Helavy, Gantar, Khadzravy, Dairi and Deglet-Nour in third group. Apart from important cultivars like Deglet-Nour with core from southern Algeria and Majoul from Morocco, according dendrogram could conclude that its distribution corresponded roughly with geographic dispersion. However, the cultivars of Khousestan and adjacent areas divided into two quite distinct groups, and Kerman and Sistan va Baluchestan

cultivars laid in a group between this tow. Because the same soil and climate, farmers of this two adjacent province have been swapping the same genetic palms. Arbnejad et al (2012) based on Nie genetic distance analysis clustered 16 cultivars into three major categories distinguished Africans, Iranians and Iraqians. Kheirallah et al (2013) divided 30 Iraqi cultivars into two major categories by bootstrap method, one of which was divided into 3 subgroups.

Analysis of molecular variance (AMOVA) showed a difference of about 5 percent between male and female palms, while internal variance was close to 95% in both groups (Table 3). Comparison of genetic variances (Table 4) also revealed there is no significant difference between male and female cultivars in terms of genetic background, and as Pournabi et al (2011) showed there is only one DNA band difference between male and female Date palms. Also in dandogram, Zahedi and Astaamran females, Verdi, Smsmavy, Ghannami and Jarvis males were with each other in one cluster also, but Rabbi and Halavy females were in opposite cluster. While studying Khierallah et al (2013) was put two versions green and yellow Ghannami in two distinct clusters on both sides and apart from females cluster. These issues show that superior males like Ghannami may have no far distance with particular females like Astaamran, or near to some of them like Halavi. Therefore, farness or closeness of the genetic distance does not cause performance of a male for a specific female. So, the more distance between males and females to choose the superior male hypotheses seems incorrect.

Mean of observed heterozygosity was 0.724 and excepted was 0.759 (Table 5). Akkak et al (2009) estimated expected heterozygosity 66%, observed 50%, Arbnejad et al (2012) showed average expected heterozygosity 72%. As observed, heterozygosity rates estimated in this study was over than previous researches that was not less than 50%. This level of heterozygosity in Date palm is unique among plants, which is caused by its dioecious nature and have maintained via somatic proliferation.

Comparison of observed heterozygosity (Table 5) showed there is no significant difference between male cultivars with 0.661±0.111 and female with 0.787±0.041. Observed heterozygosity levels were in most favorite male (Ghannami) 0.875, in Vardi and Fanouch 0.750, in Jarvis and Sabzparak 0.625, in Samesmavi and almost unknown cultivar Jalough 0.500. Heterosis superiority of the male cultivar in addition to its ability to produce more pollen, with more diverse pollen grain production allowing it to increase general combining ability (GCA) between the pollen and ovum, cause improvement in quantity and quality of the fruits. Because, Because, more heterosis in male cause greater variation in pollen, create a competitive situation that increases the possibility that from the 3 ovules ready to insemination, pollen fertilize which have a greater genetic distance. Thus, Xenia and Metaxenia phenomena in the stone and fruit level will cause increase to quality and production. Therefore, when choosing the male stock must select clones with heterozygosity rate more than 0.75.

Observed heterozygosity levels in the number of preferential trading cultivars such as Mazafati, Braim, Majool and Ashgar was 1, in Astaamran, Barhi, Khadzravy, Dglet-Nour, Dairee and Gantar was 0.875, in Shakar, Halili and Golgoly was 0.750, in Halavy, Hamravi and Zahedi 0.625 and in Almahtary, Jouzie and the Rabbi was 0.750 (Table 6). As seen, the quality and adaptability level of female cultivars will be reduced, when there is decrease in amount of heterozygosity. Also, It seems females that have more heterosis, addition to better growth and production, with more diverse ovule production, create better competitive conditions for diverse pollen grain reception. Therefore, when selecting the female cultivars also, must choose one that has greater heterosis.

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Tables

Table 1. Primers characteristics

Locus	Length (bp)	Repeat motif	Sequence 5′→ 3′	Ta (˚C)	Refr.
PDAG1006	199-250	(CT)22	F:GCCACAGGAAGCACATTTAG R:CCACACCTTAATCACAAACTCC	51	4
PDAG1005	377-450	(AG)10	F:GTATGTTCCATGCCGTTCTAC R:AGCCACATCACTTGGTTCA	51	4
PDAAG1023	213-240	(AAG)10	F:AGACGCTCACCTTGGAACTT R:ACCCCGCTCATGAATTAGG	54	4
PDAAG1025	214-250	(AAG)15-A6-(AAG)3	F:CTTCTCCACTGGCATCTTCC R:CACCCGTTGGGCATCTTA	53	4
DP169	180-220	(AAT)12	F:GCATGGACTTAATGCTGGGTA R:GGTTTTCCTGCCAACAACAT	54	7
DP172	199-240	(AGG)11	F:GGTGTTTGGGCCTATTTCCT R:GTCCCTCCTCCTCTGTCC	56	7
PDCAT14	140-160	(TC)19(TC)16	F:TGCTGCAAATCTAGGTCACGAG R:TTTACCCCTCGGCCAAATGTAA	57	2
mPdCIR044	280-332	(GA)19	F:ATGCGGACTACACTATTCTAC R:GGTGATTGACTTTCTTTGAG	47	5
F: forward and R: reveres primer					

Table 2. Diversity indices formulas and description

Na = No. of Different Alleles
Ne = No. of Effective Alleles = 1 / (Σ pi2)
I = Shannon's Information Index = -1 x Σ (pi x Ln (pi))
Ho = Observed Heterozygosity = No. of Hets / N
He = Expected Heterozygosity = 1 - Σ pi2
UHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) x He
F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)
Fis = (Mean He - Mean Ho) / Mean He
Fit = (Ht - Mean Ho) / Ht
Fst = (Ht - Mean He) / Ht
Nm = [(1 / Fst) - 1] / 4
Where pi is frequency of the ith allele.

Table 3. AMNOVA of SSR marker for Iranian male and female clones

Source	df	SS	MS	Est. Var.	%
Among Sexes	1	10.776	10.776	0.369	5%
Within Sexes	24	167.955	6.998	6.998	95%
Total	25	178.731		7.367	100%

Table 4. Compression of SSR diversity between Iranian male and female clones

Sex	df	SSWP	MSWP	F	Pr > F
Male	6	44.42857	7.404762	1.079007	0.414087
Female	18	123.5263	6.862573		

Table 5. Mean and SE over Loci for each Sex

Pop	Male		Female		Total	
	Mean	SE	Mean	SE	Mean	SE
Na	5	0.327327	6.75	0.559017	5.875	0.385951
Ne	3.83969	0.31284	5.033649	0.451091	4.436669	0.306716
I	1.437645	0.072816	1.70643	0.094511	1.572038	0.067272
Ho	0.660714	0.111109	0.786915	0.040844	0.723815	0.059458
He	0.727041	0.022797	0.787219	0.022953	0.75713	0.017451
UHe	0.782967	0.02455	0.808761	0.023412	0.795864	0.016722
F	0.099964	0.142488	-0.00782	0.06443	0.046073	0.076808

Table 6. Observed hetrozygosity of different Date palm clones

Male			Female					
No.	Clone	Ho	No.	Clone	Ho	No.	Female Clone	Ho
1	Vardi	0.750	8	Istaamran	0.875	18	Dayri	0.875
2	Ghanami	0.875	9	Ashgar	1.000	19	Zahidi	0.625
3	Smesmavi	0.500	10	Almehtary	0.375	20	Shakar	0.750
4	Sabze parak	0.625	11	Barhi	0.875	21	Ganthar	0.875
5	Jarvis	0.625	12	Berim	1.000	22	Medjool	1.000
6	Jealagh1	0.500	13	Jozi	0.375	23	Helaly	0.750
7	Fonoch	0.750	14	Khathrawy	0.875	24	Methafaty	1.000
			15	Helawi	0.625	25	Golgoly	0.750
			16	Hamrawy	0.625	26	Rabey	0.375
			17	Deglet-Nour	0.875			

Figures

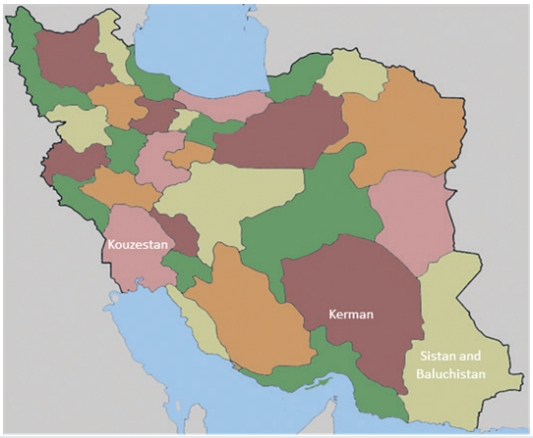

Fig 1. Map of Iranian provinces

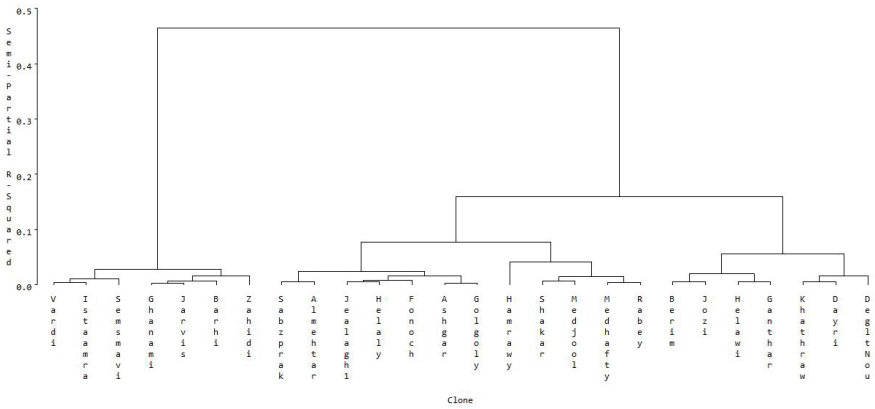

Fig 2. Dendogram of 7 male and 19 female clones of Iranian Date palm

Fruiting of Zaghloul date palms in response to foliar application of the antioxidant glutathione

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ABSTRACT

This study was investigated during 2011 and 2012 seasons to examine the effect of foliar application of the antioxidant Glutathione at 0.0, 0.025, 0.05, 0.1 and 0.2 % on fruiting of Zaghloul date palms grown under Minia region. The selected palms received four sprays.

An obvious promotion on the leaf area, total chlorophylls, leaf content of N, P, K, yield, bunch weight and fruit quality of Zaghloul date palm was observed with using Glutathione at 0.025 to 0.2 %. The promotion was associated with increasing concentrations. A slight stimulation was observed among the higher two concentrations.

Four sprays of Glutathione at 0.1 % to Zaghloul date palms was beneficial for enhancing yield and fruit quality under Minia region.

Key words: Glutathione – Zaghloul date palms – yield – fruit quality.

INTRODUCTION

Yield decline of Zaghloul date palms grown under Minia region can be solved as previously mentioned by many authors by using the important antioxidant namely Glutathione. It is the most important non- protein thiol present in the plants. It is very essential in producing antioxidant defense systems in plants that can protect the plants from reactive oxygen species. It is also important for increasing the tolerance of plants to all stresses and promoting metabolism of sulfur. Reduced Glutathione is considered the major water soluble antioxidant in photosynthetic and non- photosynthetic tissues. It is also enhances cell division and increasing the integrity of cell structure (Levitt, 1980; Rennenberg, 1982; Meister and Anderson, 1983; Dekok and Stulen, 1993; Jorge *et al.,* 1993; Foyer *et al.,* 1997; Noctor and Foyer, 1998; Tausz and Grill, 2000; Kocsy *et al.,* 2001 and Mullineaux and Rausch, 2005). Recently, Abd El-aal et al, (2012) emphasized the beneficial of Glutathione on yield as well as physical and chemical characteristics of Taimour mango fruits.

The target of this study was elucidating the effect of different concentrations of Glutathione on fruiting of Zaghloul date palms grown under Minia region.

MATERIALS AND METHODS

This study was carried out 2011 and 2012 seasons in a private orchard situated at Maghagha district, Minia Governorate on thirty 20- years old Zaghloul date palms. Soil texture is silty clay and the palms are planted at 7 × 7 meters apart. The selected palms were irrigated through surface system. Pruning was carried out to maintain leaf bunch ratio at 8: 1 (according to Sayed, 2002). Number of female spathes per each palm was adjusted to ten spathes. Artificial pollination was achieved by inserting five male strands into the female bunch using known high activating pollen source throughout 2 – 3 days after female spathe creaking followed by bagging (Omar, 2007). Each selected palm received the common horticultural practices that are already applied in the orchard except those dealing with using the antioxidant Glutathione.

The present study included five treatments from five concentrations of the antioxidant Glutathione namely 0.0, 0.025, 0.05, 0.1 and 0.2 %. Each treatment was replicated three times, two palms per each. Therefore, the total uniform

in vigour palms that selected to achieve this exported was 30 palms. Randomized complete block design was adopted. Glutathione was sprayed four times at growth start (last week of April), just after fruit setting (last week of Mar.) and at one month intervals (last week of April and May). Triton B as a wetting agent was added to all Glutathione concentrations (from 0.0 to 0.2 % at 0.05 %). Spraying was done till runoff.

During both seasons, the following parameters were carried out:-

- Leaf area (m^2) (Ahmed and Morsy, 1999).
- Total chlorophylls (a + b) as (mg/ g^{-1} F.W) (Moran, 1949 and Wettstein, 1957).
- Percentages of N, P, K and Mg in the dried leaves according to Piper (1950); Chapman and Pratt (1965) and Wilde *et al.,* (1985).
- Bunch weight (kg.).
- Yield/ palm (kg.) at the first week of September.
- Some physical and chemical characteristics of the fruits namely fruit weight (g.) and dimensions (length and width, cm.) as well as percentages of pulp and seeds.
- Pulp/ seed was also calculated, total soluble solids %, total and non- reducing sugars % (A.O.A.C., 1995), total acidity % (as g malic acid/ 100 g pulp) according to A.O.A.C., (1995); fibre crude % and total soluble tannins % (A.O.A.C., 1995).

All the obtained data were tabulated and subjected to the proper statistical analysis using new L.S.D at 5 % according to Mead *et al.,* (1993).

RESULTS AND DISCUSSION

1. Leaf area:

It is clear from the data in Table (1) that foliar application of Glutathione at 0.025 to 0.2 % significantly stimulated the leaf area of Zaghloul date palms in relative to the check treatment. The promotion was associated with increasing concentrations. Increasing concentrations of Glutathione from 0.1 to 0.2 % failed significantly to show significant promotion on the leaf area. Significant differences were recorded between most concentrations on leaf area. Treating the palms four times with Glutathione at 0.2 % gave the maximum values. Untreated palms produced the minimum values. These results were true during both seasons.

2. Total chlorophylls and percentages of N, P and K in the leaves:

As shown in Table (1), total chlorophylls and percentages of N, P and K in the leaves were significantly increased in response to foliar application of Glutathione at 0.025 to 0.2 % in relative to the check treatment. There was a gradual and significant stimulation on these parameters with increasing concentrations. Meaningless promotion was observed between the higher two concentrations. Using Glutathione at 0.2 % gave the maximum values. The lowest values were detected on untreated palms. Similar results were announced during both seasons.

3. Bunch weight and yield per palm:

It can be stated from the date in Table (2) that spraying Glutathione at 0.025 to 0.2 % four times significantly was accompanied with improving bunch weight and yield per palm rather than non- application. The promotion was significantly associated with increasing concentrations of Glutathione. A slight and unsignificant promotion on the bunch weight and yield was observed among the higher two concentrations, therefore the recommended concentration from economical point of view was 0.1 % Glutathione. The best results with regard to bunch weight and yield were obtained when the palms received four sprays of Glutathione at 0.1 %. Under such promised treatment, yield per palm reached 178.4 and 192.0 kg during both seasons, respectively comparing with the yield of the untreated palm which reached 148.0 and 147.2 kg. The percentage of increase on the yield due to using the promised treatment in relative to the check treatment reached 20.5 and 30.4 % during both seasons, respectively. Similar results were announced during 2011 and 2012 seasons.

4. Physical and chemical characteristics of the fruits:

One can state from the date in Tables (1 & 2) that treating Zaghloul date palms four times with Glutathione at 0.025 to 0.2 % caused a significant promotion on fruit quality in terms of increasing berry weight and dimensions (length & width), pulp %, pulp/ seed & T.S.S %, total and reducing sugars % and reducing total acidity %, total soluble tannins % and total crude fibre % in relative to the control treatment. The promotion was in proportional to the increase in Glutathione concentrations. Significant differences on quality parameters were observed between most concentrations except between the higher two concentrations, therefore the best treatment in this respect was the application of Glutathione at 0.1 %. Untreated palms produced unfavourable effects on fruit quality. The same trend was noticed during both seasons.

DISCUSSION

The promotive effects of Glutathione on growth, nutritional status, yield and fruit quality of Zaghloul date palms might be ascribed to its positive action on enhancing the tolerance of palms to all unfavourable conditions around the palms, uptake of nutrients especially sulfur, cell division, the biosynthesis of most organic foods and antioxidant defense systems that were responsible for protecting the

trees from reactive oxygen species (Tausz and Grill, 2000; Kocsy *et al.,* 2001 and Mullineaux and Rausch, 2005). These results are in agreement with those obtained by Noctor and Foyer (1998) and Abd El-aal *et al.,* (2012).

CONCLUSION

For enhancing growth, nutritional status, yield as well as physical and chemical characteristics of the fruits in Zaghloul date palms, it is advised to spray the palms four times with Glutathione at 0.1 %.

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Table (1): Effect of different concentrations of Glutathione on leaf area, total chlorophylls & percentages of N, P and K in the leaves, yield, bunch weight as well as fruit weight and dimensions of Zaghloul date palms during 2011 and 2012 seasons.

Concentrations of Glutathione	Leaf area (m2)		Total chlorophylls (mg/ g-1 F.W)		Leaf N %		Leaf P %		Leaf K %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 %	2.01	2.10	10.11	10.45	1.69	1.74	0.15	0.19	1.29	1.30
0.025 %	2.19	2.27	10.41	11.00	1.79	1.85	0.22	0.27	1.39	1.40
0.05 %	2.21	2.30	11.00	11.49	1.91	1.97	0.26	0.31	1.45	1.46
0.1 %	2.41	2.50	11.50	11.96	1.99	2.02	0.29	0.34	1.59	1.61
0.2 %	2.42	2.53	11.55	11.97	2.01	2.04	0.30	0.35	1.60	1.63
New L.S.D at 5 %	**0.05**	**0.06**	**0.21**	**0.31**	**0.06**	**0.05**	**0.02**	**0.03**	**0.05**	**0.05**
Character	**Bunch weight (g.)**		**Yield/ palm (kg.)**		**Fruit weight (g.)**		**Fruit length (cm.)**		**Fruit width (cm.)**	
0.0 %	18.5	18.4	148.0	147.2	21.0	21.9	5.37	5.41	2.67	2.71
0.025 %	19.6	19.9	156.8	159.2	23.3	24.1	5.50	5.55	2.72	2.75
0.05 %	20.9	21.9	167.2	175.2	25.0	25.5	5.64	5.67	2.80	2.83
0.1 %	22.3	24.0	178.4	192.0	27.3	27.0	5.70	5.74	2.95	2.99
0.2 %	22.5	24.2	180.0	193.6	27.5	27.7	5.72	5.75	2.96	3.00
New L.S.D at 5 %	**1.0**	**1.1**	**2.9**	**3.0**	**1.0**	**1.1**	**0.07**	**0.06**	**0.03**	**0.03**

Table (2): Effect of different concentrations of Glutathione on some physical and chemical characteristics of the fruits of Zaghloul date palms during 2011 and 2012 seasons.

Concentrations of Glutathione	Pulp %		Seeds %		Pulp/ seed		T.S.S %		Total sugars %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 %	77.1	77.8	22.9	22.2	3.37	3.50	26.5	27.2	20.1	20.3
0.025 %	79.4	80.1	20.6	19.9	3.85	4.03	27.6	28.4	20.8	21.0
0.05 %	81.5	82.3	18.5	17.7	4.41	4.65	28.9	29.6	21.6	22.0
0.1 %	83.4	84.1	16.6	15.9	5.02	5.29	29.8	30.5	22.0	22.9
0.2 %	83.5	84.2	16.5	15.8	5.06	5.33	30.0	30.6	22.2	23.0
New L.S.D at 5 %	**1.1**	**1.0**	**0.9**	**1.0**	**0.21**	**0.18**	**0.7**	**0.8**	**0.5**	**0.6**

Concentrations of Glutathione	Pulp %		Seeds %		Pulp/ seed		T.S.S %		Total sugars %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Character	Reducing sugars %		Non- reducing sugars %		Total acidity %		Total soluble Tannins %		Total crude fibre %	
0.0 %	14.0	14.2	6.1	6.1	0.401	0.396	0.71	0.74	0.69	0.71
0.025 %	14.6	15.0	6.2	6.0	0.380	0.371	0.60	0.62	0.58	0.59
0.05 %	15.3	15.5	6.3	6.5	0.350	0.350	0.41	0.40	0.37	0.37
0.1 %	16.0	16.0	6.0	6.9	0.322	0.318	0.35	0.33	0.30	0.31
0.2 %	16.2	16.1	6.0	6.9	0.320	0.316	0.34	0.32	0.29	0.30
New L.S.D at 5 %	**0.4**	**0.3**	**NS**	**NS**	**0.020**	**0.018**	**0.03**	**0.04**	**0.03**	**0.04**

Improving fruit quality and nutritional value of Saidy dates by using different fertilization sources

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ABSTRACTS

The beneficial effects of some fertilization treatments on fruit quality of Saidy date palm grown in sandy soil were investigated during 2004 to 2007 seasons. Various sources of nitrogen, phosphorus and potassium (NPK) such as organic, bio-fertilizers and slow-release forms of nitrogen were used compared to mineral NPK sources to determine the optimum and better source.

Amending the palms with organic form of farmyard manure (FYM) plus either potassien or rock phosphate, as well as, slow release plus either potassien and rock phosphate gave the heaviest and biggest fruits compared to fertilization with mineral sources of NPK.

The maximum fruit juice total soluble solids and sugar contents were obtained when using either organic form plus either potassien or phosphoren, or slow release-N plus potassien and rock phosphate. Also, fruit content of N, P and K were significantly increased by using organic manure, slow release-N, biofertilizers and potassien compared to fertilizing by mineral sources of N, P & K. Amending the palms with either organic plus bio-form or slow release-N gave the highest values of remaining soil-N, whereas, phsophoren or rock phosphate gave the highest value of the remaining soil-P. In addition using organic manure plus potassien significantly increased the remaining soil-K compared to other fertilization treatments.

It could be concluded that replacing the mineral requirements of Saidy date palm by either organic, bio-forms or slow release was very useful in improving the soil fertility and consequently improving the fruit traits. In addition, this procedure can reduce nitrate environmental pollution as well as maintain the soil fertility for sustainability of agricultural and organic farming production.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit crops grown in the arid regions of the Arabian peninsula, North Africa and Middle East (Chao and Krueger, 2007). Egypt is considered as the leader of Arab countries in producing dates [Food Agricultural Organization (FAO), 2009]. Dates are considered as an almost ideal food that provides a wide range of essential nutrients with many potential health benefits (Elleuch *et al.,* 2008). The dates quality can vary depending on cultivar, soil conditions, cultural practices as well as the ripening stage (Ismail *et al.,* 2006). Fertilization is one of the important tools to improve the dates physicochemical. Loss of elements of nutrients by leaching volatilization, denitrification as well as mobility of elements and other ways was the most important problem. Thus optimizing nitrogen agent loss can solve this problem. The loss of nitrogen via leaching through drainage water may be reduced to some extent by using slow release forms of nitrogen (Wang and Alva, 1996). Application of organic and chemical fertilizers were found to increase nutrient uptake and improved yield and fruit quality and decreased the fruit contents of nitrate and nitrite at both bisir and tamr stages. Increasing percentage of organic fertilizers from 25 to 75% of the recommended nitrogen rate was followed by

a gradual promotion on these traits (El-Morshedy, 1997; Shahein *et al.,* 2003; El-Assar, 2005; Badawi, 2007; El-Wasfy and El-Khawaga, 2008; El-Salhy *et al.,* 2008, Al-Kharusi *et al.,* 2009 and Marzouk and Kassem, 2011).

Moreover, the use of organic and bio-fertilization for fruit crops as good alternatives to chemical fertilization can depress environment pollution and produce a nutritive and safe food that is good for health (Blake, 1990). Using organic or biofertilization (Biogen) significantly improved the fruit quality (Osman, 2003; Mohamed and Gobara, 2004 and Mansour *et al.,* 2004).

Potassium fertilization applied to sewy date palm grown in calcareous soil increased fruit weight and TSS%, whereas, decreased the seed weight and fruit tannins content (El-Hammady *et al.,* 1991). Application of K fertilizer at two equal doses in May and December or at three equal doses in March, May and December is better. The optimum rate of economic potassium fertilization for date palms on sandy soil was 600 g of K2O/palm/year (Salama, 2007; Shahin, 2007 and Harhash & Abdel-Nasser, 2007).

Phosphorus is very important in the metabolic processes, i.e. blooming and flower development. Egyptian soils having alkaline pH are low in their availability that approximately 90-95% of P occur in an unavailable form (Olsen, 1973). Inoculation with P-biofertilizers increase phosphorus uptake by plants grown on high phosphate fixing soil (Gaur *et al.,* 1980 and Kurtsidze, 1984).

The main objective of this study is to evaluate the effect of some fertilization treatments on fruit quality of Saidy date palm grown in sandy soil. Furthermore, the possibility of using organic, bio or slow release fertilizers instead of mineral fertilizers.

MATERIALS AND METHODS

The present study was carried out during the four consecutive seasons of 2004 to 2007 at the Experimental Orchard of Agricultural Research Station that is located at El-Kharga Oasis, New Valley Governorate, Egypt.

Forty two Saidy date palms of uniform vigour 35 years old, healthy with no usual nutrient deficiency symptoms. They planted in sandy loam soil and water table depth at not less than two meters were chosen. Analysis of the soil was done before starting and after the end of study to determine the remaining soil NPK according to Wilde et al. (1985) and are shown in Table (1). The chosen palms were divided into fourteen fertilization treatments including the control. The experiment was arranged in completely randomized block design with three replicates, one palm per each. The treatments were arranged as follows:

1. Control palms received 1000 g N/palm (2.17 kg urea, 46.5%) plus 1.5 kg calcium super phosphate (15.5% P2O5) and 1.0 kg potassium sulphate (48% K2O).
2. Fertilization with 750 g N/palm as organic manure (100 kg Farmyard manure (FYM), 0.75% N).
3. Fertilization with 250 g N (33.3 kg FYM plus 1000 g Nitrobien/palm) plus 1.5 kg calcium super phosphate (15.5% P2O5) and 1.0 kg potassium sulphate (48% K2O).
4. Fertilization with 750 g N (1.9 kg Enciaben 40% N as slow release) plus 1.5 kg calcium super phosphate (15.5% P2O5) and 1.0 kg potassium sulphate (48% K2O).
5. Fertilization with 100 kg FYM plus 1.5 L Potassin-N/palm (30% K2O + 5% N).
6. Fertilization with 100 kg FYM plus 1.5 L Potassin-F/palm (30% K2O + 8% P).
7. Fertilization with 33.5 kg FYM + 1000 g Nitrobien plus 1.5 L Potassin F/palm.
8. Fertilization with 1.9 kg Enciaben plus 1.5 L Potassin F/palm.
9. Fertilization with 100 kg FYM plus 50 cm3 liquid Phosphoren/palm.
10. Fertilization with 100 kg FYM plus 1kg rock phosphate/palm.
11. Fertilization with 100 kg FYM plus 50 cm3 liquid phosphoren plus 1.5 L potassin N
12. Fertilization with 100 kg FYM plus 1kg rock phosphate/palm plus 1.5 L potassin N.
13. Fertilization with 250 g N (33.7 kg FYM) plus 1000 g Nitrobien + 50 cm3 liquid phosphoren/palm. plus 1.5 L potassin N
14. Fertilization with 1.9 k Enciaben plus 1 kg rock phosphate/palm. plus 1.5 L Potassin-N/palm

In addition, all treatments manured with 50 Kg FYM /palm

Farmyard manure (FYM), calcium superphosphate and rock phosphate were mixed and added once in a circle surrounded each palm on the middle of December. As well as enciaben as slow release fertilizer, potassin F and potassin N, as well as, Biostimulants namely nitrobien and phosphoren, were added at two equal batches on the middle of February and May. Urea was applied at three equal batches on the middle of February, May and July. In addition, potassium sulphate was added at two equal batches on middle of May and July. The data of FYM and rock phosphate analysis are given in table (1).

Other horticultural practices such as irrigation, pruning and pest control were used as usual. In addition, the artificial pollination was uniformly performed in respect of source, date and method to avoid residues of metaxenia.

In general, the following measurements were determined during the four seasons of study.

All bunches were harvested at late rutab stage and dates were picked and harvesting date was recorded. Sample of 50 fruits were taken randomly from each palm to determine of some physical and chemical fruit properties as outlined in A.O.A.C. (1985). In addition, the percentage of N, P and K in dried fruit were determined according to procedures outlined by Wild et al. (1985).

The proper statistical analysis was carried out according to the methods outlined by Snedecor and Cochran (1980) and Gomez and Gomez (1984) using L.S.D. test for distinguishing treatment means.

RESULTS AND DISCUSSION

1. Effect of some fertilization treatments on fruit quality:

Data presented in Tables (2 & 3) show the effect of some organic, bio and slow release fertilizers on some physical fruit traits of saidy dates during 2004, 2005, 2006 and 2007 seasons.

As a general view it can be noticed that all fertilization treatments were materially advanced the harvest date compared to control (T1). Furthermore, using potassien combined with either organic or bio-form advanced the harvest date about two weeks earlier as compared to NPK at mineral sources (T1).

All treatments also, caused significant increases in fruit weight, flesh weight percentage and dimensions compared to using mineral NPK only (T1). The heaviest fruits were recorded on palms fertilized with either slow release-N plus potassien-F (T8), slow release-N plus potassien and rock phosphate (T14) or organic form plus rock phosphate (T10). Whereas, the smallest ones occurred on palms fertilized with mineral source of NPK (T1).

The important role of organic manure and slow release-N in providing palms with their requirements from various nutrients as well as the positive action of these elements in the biosynthesis of organic foods and cell division (Nijjar, 1985), as well as, controlling the uptake of nitrogen by roots for a long period could give a good explanation for the present effects on the physical fruit properties. Moreover, the role of potassien in increasing the fruit weight could be attributed to the physiological effect of potassium in increasing the osmotic potential of fruit cell that might promote the water movement into the fruit, consequently increase the fruit volume and weight.

Furthermore, data in Tables (3 & 4) showed that using fertilizers, either organic, bio-form or slow release-N as well as potassien plus either phosphoren or rock phosphate were accompanied with improving the fruit quality in terms of increasing total soluble solids and sugar contents and decreasing the moisture contents compared to fertilization by mineral sources of NPK (T1).

Such, improving of fruit quality due to organic, bio and slow release N fertilizers could be ascribed to a good balance between the growth and fruiting since improved the soil fertility, Table (5) that result in accumulating more carbohydrates and makes them very available for enhancing ripening of dates.

The highest values of total soluble solids and total and reducing sugar percentages were obtained with palms fertilizeed by bio-form plus potassien and phosphoren (T13), while the lowest ones were found due to fertilization of the palm by NPK at mineral sources only (T1).

Moreover, the maximum fruit juice total and reducing sugars was obtained from palms fertilized by organic form plus potassien (T5 & T6) and phosphoren (T11 & T13) and slow release-N plus potassien and rock phosphate (T14). These finding could be related to the role of potassien on translocation of photosynthesis products in leaves. Also, phosphoren hastened the maturation of fruits, hence increase the sugar contents. In addition, the effect of organic, bio and slow release-N fertilizers on controlling the uptake of N and other nutrients by the palm for a long period and on achieving a good balance between growth and fruiting.

These results are in accordance with those obtained by Osman (2003), Shahein et al. (2003), Abdel-Hameed and Ragab (2004), Gobara (2004), Gobara and Ahmed (2004), Mansour et al. (2004), Mohamed and Gobara (2004), Abou Sayed-Ahmed et al. (2005), El-Assar (2005), Badawi et al. (2007), El-Salhy et al. (2008) and Marzouk and Kassem (2011). They concluded that Zaghloul and Sewy date fruits were improved by organic and biofertilziation. In addition, El-Hammady et al. (1991), Attalla et al. (1999), Salama (2007) and Harhash and Abdel-Nasser (2007) found that the use of potassium fertilization improved the date fruit properties.

Moreover, Table (5) showed that the fruit N, P and K contents were significantly increased by using organic manure, biofertilizers, potassien and slow release-N compared to fertilize by mineral sources of N, P & K (T1). Furthermore, using the slow release-N (T4, T8 and T14), organic manure plus potassien and either phosphoren (T11) or rock phosphate (T12) and biofertilizer such as nitrobin plus potassien (T7). In addition, bio-fertilizer plus phosphorein (T13) resulted in more announced fruit-N percentage than that fertilized by mineral sources of N, P

and K. Whereas, the other remaining treatments showed intermediate values between the two extrems. This means that natural (organic and bio-form) markedly increase fruit content of nitrogen than artificial fertilizers.

Most fertilization treatments increased the fruit phosphorus content compared to control (T1). Using either phosphoren (T9, T11 & T13) or rock phosphate (T10, T12 & T14) produced significantly higher fruit phosphorus than those with other treatments. Furthermore, using potassien significantly increased the fruit content of potassium as compared with that of remaining treatments.

These results are nearly in the same line with those obtained by Shahein et al. (2003), Al-Kharusi et al. (2009) and Marzouk and Kassem (2011). They found that the application of nitrogen in both organic and inorganic sources was preferable than using inorganic nitrogen form only in improving dates quality.

2. Effect of some fertilization treatments on remaining soil NPK:

Data in Table (5) show the effect of organic, bio and slow release-N as well as potassien, phosphorein and rock phosphate on the remaining soil NPK.

In general, fertilization with either organic manure, bio-form or slow release-N caused a significant increase in the remaining soil N compared to fertilization with the mineral source. Using slow release-N gave the highest values of the remaining soil-N in comparison with other fertilization treatments. Whereas, the remaining soil phosphorus was higher due to the application of organic manure, bio and slow release-N plus either phosphoren or rock phosphate. Moreover, using organic manure plus potassien significantly increased the remaining soil potassium compared to other fertilization treatments.

These finding emphasized the role of organic manure, bio-form and slow release-N as well as potassien, phosphorein and rock phosphate in enhancing the releasing nutrient substances from rocks in the soil and making them available to the uptake and thereby improving the soil fertility. So, such organic and biofertilization as well as the slow release treatments have a special importance for the sustainability of the soil fertility and agricultural production. These results are in partial agreement with those reported by Young (1997), Almadini and Al-Gosaibi (2007) and El-Salhy et al. (2008) who found that the fertilization of the palms with organic manures significantly improve the physical and chemical properties of soil, which on turn led to improvements in soil fertility status. They suggested that the organic fertilization practices possess a special importance for the sustainability of the soil.

CONCLUSION

According to the overall results, it can be concluded that replacing the mineral–N requirement of saidy date palms by either organic, bio forms or slow release–N, as well as potassien and phosphoren or rock phosphate would achieve a beneficial improvement of the fruit quality. In addition, these processes are very useful in saving fertilization cost and decreasing the environmental pollution problems.

These advantage will eventually enable growers to obtain high good fruit quality. Furthermore, using the organic, bio-form or slow release fertilization sources improves the soil fertility and reduces the added fertilizer requirements. Thus, the growers are able to produce organic farming products which are rellable with high price and maintain the human health.

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Tables

Table (1): Some physical and chemical characteristics of experimental soil, farmyard manure and rock phosphate used.

Soil property	Value	Farmyard manure	Value	Rock phosphate	value
Sand %	82	pH (1: 10 extract)	7.73	P2O5 %	30
Silt %	11.5	E.C (1:10 extract) (mmhos /1cm)	6.45	MgO %	0.85
Clay %	6.5	Total N %	0.75	CaO %	44
Texture grade	Sandy loam	Available P%	0.13	Fe2O3 total %	4.2
pH (1: 2.5 extract)	8.31	Available K %	1.73	Al2O3 %	0.55
E.C (1: 2.5 extract) (mmhos /1cm)	0.40	O. M. %	15.2	MnO %	0.13
CaCO3 %	4.0	C/N ratio	23.26	SO4 %	4.6
O. M. %	1.96			Na2O %	1.0
Total N %	0.13			K2O %	0.3
Available ppm (Olsen method)	2.92				
Available K ppm (ammonium acetate)	91.00				

Table (2): Effect of some organic, mineral, bio and slow release fertilizers on fruit weight (g) and pulp weight % of Saidy date palm cultivar during 2004, 2005, 2006 and 2007 seasons.

Treatment 2004		Bunch number/ palm				Bunch weight (Kg)				Yield / palm (Kg)					
		2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean		
Control palms (N, P & K as mineral)	T_1	8.78	8.65	8.83	8.72	8.75	85.42	85.55	85.39	85.55	85.48	25/9	30	22	27	26	0.0
100 kg FYM, 0.75% N	T_2	9.12	9.01	9.13	8.98	9.06	86.73	86.57	85.76	86.64	86.43	21	27	12	12	18	8.0
33.3 kg FYM + 1.0 kg Nitrobien + 1.5 super phosphatee kg (15.5% P_2O_5) and 1.0 kg K_2SO_4 (48% K_2O).	T_3	9.14	8.96	9.23	9.04	9.09	86.76	86.83	85.91	85.95	86.36	22	23	11	12	17	9.0
1.9 kg Enciaben 40% N plus 1.5 kg super phosphate and 1.0 kg K_2SO_4	T_4	9.20	9.11	9.14	9.17	9.16	86.20	85.73	86.65	85.93	86.13	18	23	16	19	19	7.0
100 kg FYM plus 1.5 L Potassin-N	T_5	9.16	9.31	9.20	9.33	9.25	86.90	86.89	86.20	86.39	86.59	19	20	9	8	14	12.0
100 kg FYM plus 1.5 L Potassin-F	T_6	9.15	9.08	9.13	9.12	9.12	86.01	86.78	86.75	86.07	86.40	18	15	10	9	13	13.0
33.5 kg FYM + 1.0 kg Nitrobien + 1.5 L Potassin F	T_7	9.24	9.04	9.19	9.16	9.16	85.93	86.62	86.29	85.92	86.19	18	18	10	10	14	12.0
1.9 kg Enciaben + 1.5 L Potassin F	T_8	9.30	9.58	9.14	9.48	9.38	85.92	86.12	86.01	86.39	86.11	16	20	11	13	15	11.0
100 kg FYM + 50 cm^3 liquid Phosphoren	T_9	9.08	9.00	9.05	9.12	9.06	86.34	86.56	86.52	86.29	86.43	20	18	8	10	14	12.0
100 kg FYM plus 1kg rock phosphate	T_{10}	9.30	9.45	9.36	9.13	9.31	86.02	86.46	86.32	86.20	86.25	17	18	10	11	14	12.0
100 kg FYM + 50 cm^3 liquid phosphoren. + 1.5 L potassin N	T_{11}	9.13	9.02	9.13	9.08	9.09	86.09	86.47	86.53	85.90	86.25	13	16	9	10	12	14.0

Treatment 2004		Bunch number/ palm					Bunch weight (Kg)					Yield / palm (Kg)					
		2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean		
100 kg FYM + 1.0 kg rock phosphate + 1.5 L potassin N.	T_{12}	9.20	9.01	9.11	8.99	9.08	86.09	86.57	85.51	86.76	86.23	17	18	10	11	14	12.0
33.5 kg FYM + 1.0 kg Nitrobien + 50 cm^3 phosphoren + 1.5 L potassin N	T_{13}	9.16	9.11	9.15	9.08	9.13	86.14	85.95	86.45	86.23	86.19	12	13	7	8	10	16.0
1.9 k Enciaben + 1.0 kg rock phosphate+ 1.5 L Potassin-N	T_{14}	9.31	9.45	9.38	9.50	9.41	86.14	86.35	86.14	86.10	86.18	17	17	8	10	13	13.0
L.S.D. 5%		0.33	0.30	0.28	0.25	0.26	0.41	0.36	0.52	0.43	0.42						

Table (3): Effect of some organic, mineral, bio and slow release fertilizers on dimension, fruit moisture % and T.S.S. of Saidy dates during 2004, 2005, 2006 and 2007 seasons.

Treat-ment	Fruit length(cm)					Fruit diameter (cm)					Fruit moisture %					T. S. S				
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean
T_1	3.44	3.41	3.46	3.45	3.44	2.01	2.02	2.08	2.09	2.05	14.25	14.50	14.68	15.00	14.61	78.30	77.60	78.50	77.80	78.05
T_2	3.55	3.55	3.53	3.48	3.53	2.04	2.06	2.12	2.10	2.08	12.67	1358	1353	13.45	13.31	81.25	79.90	80.30	80.20	80.41
T_3	3.53	3.50	3.60	3.41	3.51	2.10	2.09	2.14	2.16	2.12	13.07	13.47	14.10	13.60	13.56	80.80	80.10	79.90	80.10	80.23
T_4	3.53	3.61	3.53	3.51	3.55	2.06	2.14	2.12	2.14	2.12	13.47	13.56	13.83	13.95	13.70	79.00	78.70	79.00	78.90	78.90
T_5	3.54	3.60	3.52	3.53	3.55	2.12	2.07	2.12	2.17	2.12	12.53	12.77	13.10	13.25	12.91	81.60	81.10	80.50	80.30	80.88
T_6	3.54	3.59	3.52	3.49	3.54	2.13	2.40	2.04	2.15	2.18	12.15	12.60	12.96	13.06	12.70	81.90	81.30	80.90	80.50	81.15
T_7	3.56	3.51	3.51	3.46	3.51	2.11	2.09	2.13	2.12	2.11	12.67	12.82	13.45	13.38	13.08	81.20	81.10	80.60	80.20	80.78
T	3.59	3.61	3.46	3.70	3.59	2.23	2.07	2.17	2.19	2.17	12.70	13.20	13.26	13.00	13.04	81.30	80.80	80.30	80.50	80.73
T_9	3.56	3.43	3.57	3.43	3.50	2.08	2.12	2.08	2.15	2.11	13.33	13.50	13.07	13.43	13.33	80.60	80.20	80.80	80.04	80.50

Treat-ment	Fruit length(cm)					Fruit diameter (cm)					Fruit moisture %					T. S. S				
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean
T_{10}	3.50	3.67	3.55	3.52	3.56	2.18	2.19	2.19	2.21	2.19	13.28	13.10	13.00	13.43	13.20	80.60	80.40	81.10	80.20	80.58
T_{11}	3.57	3.65	3.54	3.59	3.57	2.08	2.15	2.14	2.13	2.13	12.27	12.66	13.42	13.30	12.91	81.80	81.30	80.60	80.50	81.05
T_{12}	3.6	3.71	3.48	3.62	3.60	2.11	2.20	2.17	2.21	2.17	12.73	12.95	13.36	13.17	13.05	81.35	80.85	80.50	80.30	80.75
T_{13}	3.56	3.51	3.57	3.60	3.56	2.07	2.05	2.16	2.16	2.11	12.87	12.20	12.61	12.65	12.58	81.00	81.70	81.30	80.90	81.23
T_{14}	3.59	3.61	3.58	3.62	3.60	2.17	2.19	2.18	2.22	2.19	12.93	13.18	13.13	13.43	13.17	81.10	80.80	80.90	80.40	80.80
L.S.D 5%	0.11	0.1	0.09	0.07	0.06	0.08	0.07	0.08	0.09	0.06	0.31	0.36	0.39	0.35	0.60	0.83	1.20	0.98	1.11	0.98

Table (4) : Effect of some organic, mineral, bio and slow release fertilizers on total sugar %, reducing sugars% and non reducing sugars % of Saidy dates during 2004, 2005, 2006 and 2007 seasons.

Treatment	Total sugar %					Reducing sugars %					Non reducing sugars %				
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean
T_1	72.8	71.43	72.95	70.95	72.03	63.80	62.48	63.90	61.70	62.97	9.00	8.95	9.05	9.25	9.06
T_2	75.65	73.67	73.87	72.60	73.95	66.75	64.57	64.73	63.37	64.86	8.90	9.10	9.14	9.23	9.09
T_3	74.82	73.67	73.40	74.17	74.02	65.9	64.86	64.05	64.07	64.72	8.92	8.81	9.35	10.10	9.30
T_4	73.50	73.53	72.85	71.60	72.87	64.66	63.59	63.53	61.80	63.40	8.84	9.94	9.32	9.80	9.48
T_5	75.95	75.13	74.27	73.25	74.65	66.78	65.93	64.87	64.05	65.41	9.17	9.20	9.40	9.20	9.24
T_6	76.13	75.33	75.27	73.32	75.01	66.63	66.10	65.07	64.18	65.5	9.50	9.23	10.20	9.14	9.52
T_7	75.46	74.50	74.38	73.20	74.39	65.92	65.9	64.98	64.25	65.26	9.54	8.60	9.40	8.95	9.12
T_8	75.33	74.50	74.15	73.13	74.28	66.16	65.47	64.72	64.27	65.16	9.17	9.03	9.43	8.86	9.12
T_9	74.90	74.30	74.50	73.58	74.32	66.67	65.17	65.00	64.32	65.29	8.23	9.13	9.50	9.26	9.03

Treatment	Total sugar %					Reducing sugars %					Non reducing sugars %				
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean
T_{10}	75.65	74.10	74.78	73.38	74.48	66.60	65.33	65.15	64.08	65.29	9.05	8.77	9.63	9.30	9.19
T_{11}	76.07	75.15	74.28	73.62	74.78	66.87	65.88	64.95	64.88	65.65	9.20	9.27	9.33	8.74	9.14
T_{12}	75.10	74.90	74.20	73.26	74.37	66.76	65.35	64.64	64.46	65.3	8.34	9.55	9.56	8.80	9.06
T_{13}	75.23	76.00	75.15	73.70	75.02	66.33	66.20	65.98	65.12	65.91	8.90	9.80	9.17	8.58	9.11
T_{14}	75.40	74.50	74.64	73.97	74.63	66.38	65.87	65.10	64.67	65.51	9.02	8.63	9.54	9.30	9.12
L. S.D 5%	1.56	1.45	1.63	1.25	1.38	1.33	0.75	0.89	1.28	1.02	N.S	N.S	N.S	N.S	N.S

Table (5): Effect of some organic, mineral, bio and slow release fertilizers on fruit-N %, fruit -P % and fruit-K % of Saidy dates during 2004, 2005, 2006 and 2007 seasons and remaining soil (RS), N, P and K at the end of the experiment.

Treatment	fruit-N %					fruit -P %					fruit -K %					RS-NPK		
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	N %	P ppm	K ppm
T1	0.49	0.48	0.51	0.54	0.51	0.108	0.104	0.126	0.126	0.116	0.93	0.87	0.92	0.96	0.92	0.13	2.92	91
T2	0.52	0.51	0.54	0.58	0.54	0.101	0.097	0.112	0.113	0.106	0.93	0.89	0.95	0.93	0.93	0.20	2.58	103
T3	0.55	0.54	0.57	0.61	0.57	0.106	0.112	0.127	0.128	0.118	0.94	0.89	0.94	0.96	0.93	0.19	2.95	85
T4	0.59	0.58	0.62	0.66	0.61	0.111	0.110	0.126	0.132	0.120	1.00	0.95	1.02	1.03	1.00	0.23	2.89	86
T5	0.56	0.55	0.59	0.62	0.58	0.118	0.097	0.119	0.120	0.114	0.98	0.98	1.04	1.06	1.02	0.20	2.74	120
T6	0.54	0.54	0.57	0.59	0.56	0.115	0.112	0.126	0.128	0.120	1.03	0.98	1.06	1.08	1.04	0.19	3.08	112
T7	0.57	0.56	0.6	0.63	0.59	0.110	0.106	0.123	0.129	0.117	0.99	0.95	1.05	1.07	1.02	0.19	2.93	117
T8	0.61	0.59	0.63	0.67	0.63	0.113	0.120	0.13	0.128	0.123	1.05	0.96	1.05	1.11	1.04	0.21	3.10	103
T9	0.56	0.55	0.59	0.63	0.58	0.158	0.148	0.153	0.163	0.156	0.96	0.92	1.01	1.01	0.98	0.22	3.61	102

Treatment	fruit-N %					fruit -P %					fruit -K %					RS-NPK		
	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	2004	2005	2006	2007	Mean	N %	P ppm	K ppm
T10	0.55	0.54	0.57	0.59	0.56	0.151	0.150	0.154	0.171	0.157	0.96	0.91	0.97	1.00	0.96	0.22	3.57	107
T11	0.57	0.56	0.61	0.64	0.60	0.144	0.135	0.167	0.170	0.154	1.05	0.99	1.06	1.10	1.05	0.20	3.44	122
T12	0.58	0.56	0.6	0.63	0.59	0.153	0.133	0.151	0.151	0.147	0.99	0.96	1.05	1.06	1.02	0.20	3.24	124
T13	0.56	0.57	0.59	0.63	0.59	0.151	0.148	0.160	0.181	0.160	1.04	0.98	1.06	1.08	1.04	0.23	3.53	118
T14	0.59	0.59	0.61	0.63	0.61	0.151	0.148	0.156	0.173	0.157	1.04	0.98	1.03	1.06	1.03	0.25	3.70	116
L.S.D.	0.05	0.04	0.03	0.04	0.03	0.008	0.007	0.010	0.009	0.010	0.07	0.09	0.08	0.07	0.09	0.05	0.38	11

An analytical study of the first national food product from date palm products in Palestine (PALMIX)

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ABSTRACT

The beginning was an initial experiment end of 2012 through the production and marketing department at Omega. for Consultation &Development Co to produce the first food and patriotic product depends on date palm products with the addition of some of natural herbal and plant oil. The product went through several experiments, field studies, and laboratory analysis locally and regionally to be registered as national product by official bodies the Gaza Strip.

The studies and laboratory analysis have proven in both food analysis center in the Al-Azhar University – Gaza, and National Institute for Nutrition Researches in Egypt after taking random samples from the product(5-10) samples, the size of the sample bottle was 250 gm., then laboratory tests procedure accomplished "physics, chemises, and microbiology" for the samples and the results were: (Energy 347.9 - 438 k.cal), (Carbohydrate 65.16 - 77.12%), (Fat 8.2 - 12.48%), (Protein 3.44 - 4.24%), (Calcium 45 - 47 mg/100gr), (Potassium 511mg/100gr), (Ash 1.58 - 1.74%), respectively.

The results of Heavy Metals Report which is carried out by Public Health Lab (MOH) also showed that (Zinc 26.73 mg/Kg), (Total Iron 24.6 mg/Kg), (Sodium 0.45 g/Kg), (Potassium 3.11 g/Kg)

The Microbiology test results of samples also showed the product is totally free from any Fungi, Yeasts and Pathogenic Bacteria, these results adopted from laboratory of Palestinian Ministry of Health, Supply Ministry and Customer Protection Gaza Strip.

The field studies also for the product showed that it's lead to heal from many diseases, especially anemia because it contains iron, boron and vitamin "A" in addition to its impact on the stomach diseases and digestive tract because it contains vitamin "A" and "B1" in addition to the protection from osteoporosis because it contains calcium by high degree, it also Refurbished for blood circulation and increase the sexual energy to its contain of zinc and some of vitamins and it's useful in cases of patients with heart disease, especially high blood pressure, it is also considered as full food for Pregnant women and breastfeeding, it turns women hormonal activity back after childbirth.

We conclude from these studies and laboratory test that the product (PALMIX) is full food product to all family members as it contains many vitamins minerals, and compounds which are important for the body, energy-rich food product and strong general tonic and good food for children and appetizing and it's tonic for memory,

The product is free from any chemicals or any preservatives, there is no side effects or any negative impacts specially for people with diabetes.

This study has recommended the implementation of research and field studies on anaemic patients, especially children.

Keywords: date palm product, analysis, diseases.

INTRODUCTION

Because of its importance long ago Nutrition and Food issue still worries many countries of the world. Increased reports were issued by the United Nations around widespread hunger and wars in the Third world especially after the spread of wars & conflicts of Africa, Asia and the Middle East even though it has fertile lands and owns several sources that qualify it to be in the forefront of countries but wars and conflicts on those resources lead peoples to pay cost of the wars.

According to the nutritional status in the Gaza Strip, we find the worst because of siege, closure, and systematic destroying for agricultural lands continually, and not being able for accessing to food recourses although of existing feeding programs which applied by UN. In the Palestinian camps, there are 44% of children in Gaza are suffering from anemia. (the Nutritional status in Palestine (Isaac.J.,et.al 1995) Kanoa,B.,J, Hamed,T., Zabut,B.M (2011). Radi S.M (2010)

Few plant species have developed into an agricultural crop so closely connected with human life as the date palm's has. One could go as far as to say that, had the date palm not existed, the expansion of the human race into the hot and barren parts of the "old" world would have been much more restricted. The date palm not only provided a concentrated energy food, which could be easily stored and carried along on long journeys across the deserts. El-Sohaimy S.A. and Hafez E.E.(2010)

"Palmix" depend on many components and materials in its main composition which have directly benefit on human health because these materials contain nutrients, vitamins and minerals, especially dates products, which represent 70% of the composition of the product as main component, palm paste has been used as it contain many benefits to human body, and molasses-(dibs) which provide body with high energy and vitamins in addition to the usage of pollen because of its contain minerals and proteins and vitamins, with high-value, which is used to treat many cases of sexual dysfunction, infertility and others., Hassan, H.M.M (2011) Ganbi.H.H.A,(2012).

The fruit of the dates are good sources of sugars, vitamin C, provitamin A, of minerals and fibers. Its output in flesh added to its biochemical features, destine it to several potential technological transformations in the domain of food science, Sadiq,I. S (2013) Al-Jubouri, H.J ., Zaid, A (p 422-427). Date Palm Product (p125 -158) FAO.

The dates can be considered as a fruit, food, medicine, and as sweet. It can be an ideal and adequate food for humans because of containing main materials like sugar, amino acids, minerals, fats and proteins, and others. The Dates have significant therapeutic value as it contains antioxidants and sex steroids to contain the element phosphorus and boron and zinc.

Eating only 100 gm of dates, provide the body with full daily needs of each of magnesium and manganese, copper and sulfur, half of its iron, and quarter of its calcium and potassium. Dates contain high percentage of vitamin A and the total of vitamin B, especially vitamin Pripuflavin and Althiasan and an important source of folic acid. Nutritional and therapeutic value of Dates .rudyman,Kh (2003), El- sohaimy,S.A.et al (2010. In addition to using olive oil with 8% as its many benefits on health, especially for heart and stomach patients, especially breast cancer and so on, and as it contains amino acids and phenolic compounds- La Lastra, C et at (2001)

It has spread in recent times the use of alternative medicine using herbs nature as were used in the past prior to their use of drugs, which have sparked offenders often Plants Medical have many benefits are used to treat a variety of ailments and difficult cases and other grasses Natural does not have any side effects if used in a way correct and appropriate dosages. Rakshit, M and Ramalingam, C (2010)

Palmix also depend on medicinal herbs, and aromatic plants, which represent 12% of the product composition because of its many benefits, and for the treatment of many diseases. Spices constitute an important group of agricultural commodities which are virtually valuable in the culinary art. In India, spices are important commercial crops from the point of view of both domestic consumption and export. Besides, huge quantities of spices are also being consumed within the country for flavoring foods and are also used in medicine, pharmaceutical, perfumery, cosmetics and several other industries. There are over 80 spices grown in different parts of the world and around 50 spices are grown in India. The spices that India can offer in abundant quantities are pepper, ginger, turmeric, chilli, cardamom, celery, fenugreek, fennel, cumin, dill, coriander, cinnamon, ajowain (bishop's weed), cassia, clove, nutmeg and mace. Major spices of export are pepper, cumin, cardamom, ginger, turmeric and chillies. Other minor spices include ajowain, aniseed, celery seed, caraway, fennel, fenugreek, coriander, garlic, onion,

saffron, vanilla etc. Among the spices exported, pepper has the leading position in terms of both quantity and value realised. Beside this spices have a great potential to be used either for production of natural antibiotics and food preservatives. Almost every spices have some antimicrobial activity against human, food or plant pathogen even spices like turmeric have been used for preservation of organs. They also exhibit antioxidant property. Thus in future extensive research can be done to customise the multi-usage of spices in various fields. - Rakshit,M and. Ramalingam, C. (2010)

The Department of Nutrition for Health and Development, in collaboration with FAO, continually reviews new research and information from around the world on human nutrient requirements and recommended nutrient intakes. This is a vast and never-ending task, given the large number of essential human nutrients. These nutrients include protein, energy, carbohydrates, fats and lipids, a range of vitamins, and a host of minerals and trace elements.

Many countries rely on WHO and FAO to establish and disseminate this information, which they adopt as part of their national dietary allowances. Others use it as a base for their standards. The establishment of human nutrient requirements is the common foundation for all countries to develop food-based dietary guidelines for their populations.

-Passmore, R ., Nicol,B.M., Rio,M,N (WHO 1974)

From this point has been the adoption of the components of this product, which consists of palm products because of its many benefits in addition to natural herbs to suit the needs and requirements of human food daily.

MATERIAL & METHOD

This study was conducted for the first food and national product at the end of 2012 “Palmix”, through the production and marketing department at Omega Company – Gaza. This product is made with 70% of the palm products ***(date palm paste, date palm syrup, date palm pollen grain)***, in addition to the 8% of olive oil, 12% from natural herbs (***Nigella sativa - fennel - anise - cinnamon – Sesame*** and other herbs) 10% of the vegetable seeds and other nuts.

The aim of this study is to scoop mineral elements and compounds active in the new food product and its impact on human health. The product does not contain any chemicals or preservatives material..

Analytical methods

The first phase, where 5 samples of the product were taken randomly (sample size of 250 g glass) and the samples were sent to the food analysis center at the Al-Azhar University - Gaza, also a 15 random sample were taken to the National Institute of Research and Nutrition _ Egypt in order to conduct laboratory tests (chemical, physicists Microbiology) including: (energy, carbohydrates, protein, fat, moisture, calcium, potassium, ash)

Also in this phase 5 samples were sent to the laboratory of the Ministry of Health, Gaza in order to analyze microbiology and heavy minerals like manganese, iron, phosphor, zinc, and sodium.

- Estimating of Carbohydrates by using a Spectrophotometer at wavelength (490 nm(.
- Estimating of energy = (g protein x2.44)+(g lipid x carbohydrate x 8.37 x 3.57)
- Estimating of Proteins by using the Kjeldahl method on the basis of Nitrogen
- Estimating of Calcium, Zn – F –Mn – K – Mg. by using Atomic Absorption.
- Estimating of Potassium by using Flam photometer.
- Fat content: extract samples through the extraction device using a mixture of Chloroform/Methanol, (2 : 1.)
- Ash: through using the device of Muffle furnace.
- %Moisture content : $\frac{\text{M. intial} - \text{M, dried} * 100}{\text{M. intial}}$
- % Total Solids = M. Dried / M.initial *100

And in the second phase where field study has conducted through distribution of the product in the form of a random sample of patients, such as, sexual Weaknesses (40 study sample) and, Diseases of the stomach and digestive tract (10 S), and heart diseases (10 S) this was accomplished through pharmacies which existed in the area or individual cases.

In addition, this samples were distributed to group of children from 5 years to 12 years (40 child) in order to know the effectiveness of the product on the rate of activity, memory, and appetite

RESULTS & DISCUSSION

First Stage

Energy and carbohydrates and chemical elements:

Evidenced by the results of laboratory analysis of the product in the table (4), that shows the nutritional value of the product “Palmix” compared with nutritional value of dates and some varieties of other fruits table (1), we find that the product contains a high rate of energy (348 - 348 k Gal) average 393 k Cal, carbohydrates (65-77.12) average 71.1%, while the result of protein was (3.44 - 4.42) and average 3.43%, fat (8.2 - 12.48) average 10.34%.

While we show that the nutritional value of fruits and dates in the table (2) It's less than what is shown in the product, as the protein 2.2, energy 274 k Cal, 0.5 fat, sugars 72.9 gm.

We conclude from this study .table (7) that the product contains a high rate of energy, protein and fat, since it include 17-20% of the energy and 10% of the proteins that the common human requirement, and the percentage of fat in the product is high because it contains olive oil, some important herb, dates syrup "dibs".

Mineral elements and vitamin:

Due to not being able to measure some of the elements and vitamins in the Gaza Strip due to closure and the lack of capabilities in the local laboratories, so the items available in dates syrup and pollen and paste of dates were approved, especially which is related to vitamins, phosphorus and magnesium and manganese as it shown in the tables (3,8,9,10).

The results of the laboratory analysis of the product as in the table (5). It contains calcium 47 mg / 100mg, potassium 511 mg / 100mg, zinc 2.67 mg/100g, iron, 2.46 mg/100g sodium 0.45mg /100gm, . And by comparing these results with the mineral elements of dates in the table (3) we find that the product is almost equivalent to the mineral content of dates in terms of mineral elements as the date contain, mg / 100 g : calcium 59 mg, phosphorus 63mg, magnesium, potassium 648 mg.

With comparing these result with body requirement, we find that the product provide the body with 25-30 % of its needs of potassium, about 7-10% of the calcium.20 -25% iron, and about 25% of the zinc table (6)

We conclude from this study that the product contains several kinds of minerals, vitamins that human need every day with varying percentage, because it contains (pollen gains, herbs, vegetables seeds) which involved in the production process

Second Phase

The Effect of taking the product on samples Sexual Dysfunction and Infertility:

Reference to the segment "samples" in the table (11) observed that about 75% of those who suffer from Sexual Dysfunction and infertility gave them good results and this resulted from the presence of zinc and boron and some herbs relevant and about 25% - 30% differ from sample to another by associated factors with this side effect of taking the product samples Sexual Dysfunction and infertility.

The Effect on Diseases of the Stomach and Digestive Tract and Intestines samples:

Near 85% of people who suffer from stomach diseases and digestive tract and intestines specially dyspepsia we find good result for this sample.

The Effect of Product on Heart Diseases Samples:

Those who suffer from heart disease, especially blood pressure has been observed that there is average response rate estimates to 60% of the samples and may be due to some other reason needs to be studied

The Effect of the Product on Children Samples (Anaemia, Activity, and Memory):

For segment of children still need study special cases of anaemia and memory

We conclude from this study that the product helps to treat cases of disease with varying percentages, especially with regard to sexual dysfunction, stomach disease and intestines, but need many specialized scientific studies on the Heart Diseases, Children

Note: Percentages shown above are considered estimated

Microbiology Analysis:

Observed from the analyzed samples which sent to laboratories Ministry of Health and of National Economy table (12) that the samples are free of pathogenic bacteria, yeasts, chloroform molds and E.Coli, and this product is fit for consumption without any healthy obstacles. We conclude from this study that the product Palmix is a full healthy and good dietary product for all family member and provide the body with energy and vitamins and minerals needed for growth.

CONCLUSION

(PALMIX) product is food full product to all family members as it contains many vitamins and minerals, and compounds which are important for the body, energy-rich food product and strong general tonic and good food for children and appetizing and it's tonic for memory, the product is free from any chemicals or any preservatives, there is no side effects or any negative impacts specially for people with diabetes.

This study recommends the implementation of many of the studies, especially for children who suffer from anaemia in the Gaza Strip.

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Tables

Table (1): Nutritional value of the Dates, compared with some types of fruit:

Fruit types	Energy Contains	Protein Contains	Fat Contains
Dry Dates	233	2.4	.4
Semi-dry Dates	156	1.2	.3
Wet Dates	78	1.0	.4
Dates without nuclei	274	2.5	.5
Apple	49	.3	.2
Fig	75	.8	.2
Dates Syrup	386	0	0
Grape syrup	258	.7	.1

Table (2) : Chemical Elements of the Dates (100g/)

Elements	Content %
Water	22.5 %
Energy	274 Calories
Protein	2.2mg
Fat	.5 mg
Sugar	72.9gm
Crude fiber	2.3 gm
Ash (Mineral Elements)	1.9 gm

Table (3): Mineral Elements and Vitamins /100gm

Elements	Content %
Calcium	59 mg
Phosphor	63 mg
Iron	3 mg
Potassium	648 mg
Vitamin A	50
Thiamin	.09 mg
Pripuflavin	.10 mg
Thiasin	2.2 mg

Table (4): Palmix product Component Analysis:

Elements	T1	T2	Average
Calleries	348	438	393
Carbohydrate	65.11	77.12	71.1
Protein	3.44	4.42	3.93
Fat	8.2	12.48	10.34
Ash	1.58	1.47	1.52
Moisture	21.67	4.51	13.08

T1 - Al-Azhar Lab T2 - Egypt Lab

Table (5): Mineral Elements & heavy metals /100gm of Palmmix

Elements	Content %
Calcium	47 mg/100g
Potassium	511 mg/100g
Zink	2.673 mg/100g
Iron	2.46 mg/100g
Sodium	.45 g/kg 45 mg /100g

Table (6): Dietary Recommendations of Mineral Elements:

Elements	Children (7-10) years	Recommendation Dietary allowance mg/day		Pregnant and breast feeder women
		Male	Female	
Na	400	500	400	500
Calcium	800	800	800	1200
Magnesium	170	350	280	355
P	800	800	800	1200
K	1600	2000	2000	2000
Iron	9mg	10-15mg	10-12	15
Zn	5-8 mg	11	8	8

Table (7): Dietary Recommendations of chemical Elements

Elements	Requirements
Carbohydrates	6 – 10 gm/kg from body weight
Energy	24 – 40 k Cal /body weight
Protein	1.2 – 1.4 gm /kg – body weight
Fat	15 – 20% from energy

Handbook on Human Nutritional Requirements, FAO/WHO 1974

Table (8): Proximate chemicals composition (g/100g dry weight) of palm pollen grain

Parameter	Palm pollen grains
Moisture (%)	28.80
Ash (%)	4.57
Grude fiber	1.37
Grude fat	20.74
Grude protein (%)	31.11
Carbohydrate (%)	13.41

Values are means of three replicates.

Table (9) : Vitamins composition of palm pollen grains:

Vitamins	Palm pollen grains
A (IU/100 g)	7708.33
E (IU/100 g)	3030.92
C (mg/100 g)	89.09

Values are means of three replicates.

Table (10): Mineral composition (mg/ 100g dry weight) of Palm pollen grains

Mineral	Palm pollen grains
Boron (B)	309.4
Zink (Zn)	281.0
Selenium (Se)	305.0
Iron (Fe)	241.0
Molybdenum (Mo)	302.2
Copper (Cu)	319.6
Manganese (Mn)	284.0
Cobalt (Co)	305.5
Nickel (Ni)	302.4

Values are means of three replicates.

Sources Table (8-9-10-): Global Journal of Biotechnology & Biochemistry 6 (I): 01-07, 2011

Table (11): The effect of the product on diseases

Cases	No of sample	Result %
Sexual Dysfunction and infertility	40	75 %
Diseases of the Stomach and Digestive Tract and Intestines	10	85%
Heart Diseases Samples	10	60%
Children Samples (Anemia, Activity, and Memory	40	NA

Table (12): Microbiological analysis

Test	pathogenic bacteria	yeasts	chloroform	molds	E.Coli,
Result	Neg	Neg	Neg	Neg	Neg

Analytical study of the competitive situation of the Algerian dates in the international markets

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ABSTRACT:

Dates are considered among Algeria's main exports crop as the average value of dates exports has totalled approximately 45 million dollar annually and represents 75% of the average total value of agricultural and food exports during the period 2002-2010.Algeria is also one of the main dates-producing countries in the world, with average production of dates 7% of the average total world production during the same period.

Actually, it has been found that Algeria is ranked seventh in terms of quantity among the dates exporting countries in the world; its exports represent 3.5% of the international export capacity of dates. The contribution of the Algerian dates exports in world trade is considered limited when compared to the most important exporting countries of dates.

This research aims to analyze the competitive situation of the Algerian dates in the most important international markets. This required studying the evolution of the relative importance of exports of dates in Algeria to local production, the total value of exports of food and fruits, the geographical distribution of exports of dates according to the groups of the states, the evolution of the quantity, prices and value of dates exports, the market share for Algerian dates exports in the most important international markets in 2006, the rates of markets penetration of the main importing countries, the important competitor countries, the competitive position of the Algerian dates in those markets, and proposing ways which can be followed for improving the competitiveness of Algerian dates in world markets.

Key words: competitiveness, markets, production capacity, export

INTRODUCTION

The dates are considered among the export crops in Algeria, where exports amounted about 13000 tons, representing 3% of the world export capacity for dates and estimated its value about $ 45.8 million dollars annually, representing 71.85% of the average total value of agricultural and food exports during the period 2002-2007.

The research problem is in the competitive weakness of the Algerian dates in the important world markets where the exports of dates do not contribute only 2.26% of the average total amount of world exports of dates and about 4.7% of the average total value of world exports of dates during the period 2002-2007.

This study aims to analyze the competitive situation of the algerian dates compared with the most important competitor countries in the international market, in addition to that, it has been identifying the strengths and weakness of Algeria in the dates international markets. In addition to identify the promising markets for algerian dates in the future and the possibility of its efficiency.

METHODOLOGY

The study is based on the descriptive and statistical analysis such as trends, and regression, as well as some important economic indicators such as the comparative advantage, market share, prices competitivity, markets penetration, it has also been analyzing the competitivity of Algerian dates exports in the most important external markets.

The study basec on the secondary data from the local level, such as the agriculture ministry and the international level, such as www.faostat.org and www.comtrade.un.org.in

RESULTS

The most important found results of the study:

1. Production capacity:

The dates are considered among the important agricultural crops in Algeria and the date production of Algeria attain about 644 740 tons in 2010 and mentioning that the production capacity of dates primarily depend on all of the area planted with palm trees and the total production and average productivity of this crop.

1-1. The area planted with palm trees:

The cultivated area by palm is took general trend upward during the period 2000-2010 Table (01) in Annex, where the total planted area with palm trees in the year 2010 about 161 090 hectares, an increase of 58.21% from the base year 2000. The reasons of this increase to the reforms defined in the agricultural sector in that period, especially the application of the law 83/18 of 13.08.1983 relating to the acquisition of agricultural real property (APFA), In this process important agricultural areas are distributed to beneficiaries for reclamation process, in addition to the refurbishment of palm trees.

1-2. The evolution of dates productivity:

Table number (01) in Annex shows evolution of productivity dates during the period from 2000 to 2010, where it was known by general trend upward during the study period and reached a maximum of 50.5 kg in 2010, an increase of 23% from the base year. Spite of average productivity of Palm 46.82 kg / Palm during the period 2000-2010 AD, however, this average is still far from the world average. And the average production arrive to 100 kg / palm in (Phoenix and Alerazona) oasis in United States of America, and 98 kg / Palm in Egypt and 80 kg / Palm in Palestine.

1-3. The evolution of the dates production:

In examining the evolution of the dates production during the period from 2000 to 2010 noting that this development in the production knews a general trend upward, the dates production has reached in the year 2010 about 644 740 tons, an increase of 76.34 % from the base year 2000. This increase is due to a number of factors, the most important, stopping of economic development and service since 1986 and the growing numbers of unemployed engendered opposite emigration and the return of large numbers of labor to work in the oasis and the caring again the sector of palms [4] and it has been estimating general time trend equation of productivity during the period under study (equation No. 02, table 02) . And can be seen from the equation that the dates productivity has taken an upward trend rate of annual increase but not significant statistically amounted to 0.37 kg / Palm represents about 0.6 % of the average productivity during the same period, which amounted to 46.82 kg / Palm

The total production of dates:

The total dates production determined by influencing factors on both the cultivated acreage and average total production. it has been estimated the relation between the total production of dates and the total cultivated area during the period 2000-2010, which took a form of the following function Yi = 507+23297xi

Where : yi = estimated value of the total production of dates in tons per year i
xi= variable time i- where i = (1,2,3 ..... 11)

The equation indicates that the cultivated area explain about 91 % of the changes in the total production and the rest is attributable to productivity and that did not demonstrate a significant increase .

It is clear also from Table(1) in Annex that total production has escalated at a rate of annual increase statistically significant at 0.05, 0.01 ratio of 6.2% from the average for the period 2000-2010, which is about 499 652 tons, and it has been estimated the general time trend equation for total production (equation 3 Table(02) in Annex)

2. Foreign trade:

This section examines the relative importance of dates exports within the agricultural exports, as well as the status of Algerian dates exports among the most important exporting dates countries in the world, it will also examine the most important world markets for Algerian dates in terms of quantity and prices, and the future of Algerian dates exports to those markets.

2-1. The evolution of the relative importance of Algeria's dates exports from agricultural exports:

Actual data from the table (3) in Annex Figure 01: note

- Date exports have recorded the highest value in 2007 that exports increased more than 56% amounting to 230.83 million dollars due to the high quantity of exported dates, which amounted to 13 356 tons, and then it declined gradually until it reached 13.698 million dollars in 2009 by evolution ration amounted 92.88%.

- The proportion of the dates exports value to agricultural exports ranged between 23.54% in 2008 and 5.02% in 2009 and the decrease is attributed to the high proportion of domestic consumption of dates (90%).

2-2. The most important dates exporting countries:

It is expected to increase the demand on dates in the world in the future, in light of the efforts to publicize its food benefits and health, which led to the developed

uses of dates in European countries and the global countries in the domain of food and medical industries.

It is clear from Table (4) in the Annex that shows the order of Algeria among the world's dates exporters, we find that the average Algeria's dates exports amounted to 12931 tons, 2.26% of the average total international date exports, and thus it comes in sixth place after the United Arab Emirates, Iran, Pakistan, Tunisia, and Saudi Arabia.

While the average value of its exports 18171 thousand dollars and form 4.7% of the average total value of world dates exports during the period 2002-2007.

It is clear from this that the amount of Algeria's dates exports for the value is considered as low than the dates exported from the other countries, where dates exports in Tunisia is 8.19 % of the total international dates exports, and 25.20 % of its value, followed in the importance Iran by 24.21 % from the total international date exports and 14.75 % of its value, then Israel 1.4% of the total international date exports and 9.46 % of its value . Clear from these data that there is great variation in terms of revenue per ton of dates according to the source, the rate price of ton of Tunisian dates approximately 2077 dollars, the rate per ton of Algerian dates amounted to $ 1405 . For comparison the average price of exported dates from the United States is 2664 dollars, and Israel 4570 dollars.

Concludes from the foregoing that the Arab countries produce large quantities of dates, but that the rate of export is very weak, reaching 7.26 % Average years from 2002 to 2007 at the same time most of these dates are sold by low prices compared to production of other countries.

2-3. The evolution of Algeria's dates exports during the period 2000-2010

From Table (5) in Annex Figure (2), which shows the evolution of the value of Algerian dates exports and its quantity during the period from 2000 to 2010 it is shown as following:

The value of Algerian dates exports get evolved by 114.8 % during the years 2000 to 2010 where it was 14748 thousand dollars in 2000 increased continuously until it reached its maximum of 23 083 thousand dollars in 2007, augmentation about 56.62 % from the base year 2000, but decreased in the following year to 20013 thousand dollars because of the decrease of the quantity from about 13356 tons in 2007 to about 10055 tons in 2008.

In terms of the total weight of the Algerian exports of dates and from the table (5) in Annex.

We note the fluctuation in the quantity of Algeria's dates exports during the period (2000 - 2010), and it has reached its maximum in 2007, reaching 13356 tons and reached a minimum in 2004, reaching 2585 tons, The quantity dates exports have taken a general trend decreasing during the study period, due however, that the most of the dates production is consumed internally .

2-4. Geographical distribution of Algeria 's total dates exports:

The data of tables (6) and (7) in the Annex indicates that the European countries are the most important importing countries of Algerian dates, where the average quantity exported to them is about 9557 tons, about (87%) of the total exported quantities of dates and at average price of 1627.9 DA/ton

Also, it is noted that the imports of European countries of Algerian dates increased during the period 2002-2007 from 9864 tons in 2002 to 11094 tons in 2007, meaning that the percentage of the exports quantity to these countries increased from 89.9 % to 101% during the same period and France imported during the same period 8280 tons means (86.91 %) of the total exports to European countries, and Belgium comes in second rank with 551 tons (5.8%).

And American States come in second rank, they import 5.4 % of the total exported quantity and it constitutes 6.85 % of the total quantity of exported dates and at average price of $ 2092.9 per ton.

We conclude that Algeria's exports to the U.S. markets have reached its minimum of about 114 tons in 2006, about 0.92% while it reached its maximum of about 1142 tons in 2007, about 8.5 %, and the Canada's imports represent during the period from 2002 to 2007 an average of about 470 tons, about 60.4% of total exports to the American States, as the United States imported the rest (34.3%) in that year (Any 237 tons) and the both Canada and the United States of America considered as new markets for Algerian dates, were discovered newly after the year 1993 .

And the African countries occupied the third rank; they import 4.1 % of the exported quantity and constitute 5.2% of the total quantity of exported dates and at an average price of $ 213.8 per ton.

The date exports to African countries have reached its minimum of about 137 tons in 2004, while the maximum of about 706 tons in 2007 and Guinea obtained (3.12%) of Algeria's exports to these countries during the average period of 2002-2007 and (0.99%) to Mali and the majority of the exported qualities to these states are dry dates unfit for improved squeeze

• Exports to Asian countries are relatively weak, especially in recent years.

Exported dates Prices vary greatly from a country to another, and this difference in price is due mainly to the difference in exchange rates and to the exported varieties.
And It is observed that most of the exports of dates moving towards the European Community and in particular France due to the low transportation costs and to the attractiveness of these markets, and France trends to import the dates at the end of each calendar year, it imports 90% of its imports during the months of October and December, and it is observed that these periods coincides with the harvest periods in Algeria, while the rest of dates exports are distributed between the Asian countries and some European countries and African and American countries, and Arab countries in small and varying rates.

2-5 Sharing Market of the Algerian dates exports in the most important international markets:

The data in table (08) in Annex indicates to the lack of market part of Algeria's dates exports in some of the most important world markets (0.25% of the German market, 0.22% of the Spanish market, 0.11% of the Italian market) and not presented in others (Switzerland – India)

2-6 Market Penetration rate of the most important dates importing countries:

The rate of market penetration is known as the ratio between the states imports of the goods and the actual consumption of the same goods, and whenever that ratio increased means the breadth of the market and the easiness to enter it and vice versa, and the data of table number (09) in annex indicate to rising of the rate indicator of the market penetration of the main dates importing countries in 2006, the value of this indicator reached the maximum in the French market reaching 1.72, and reached the minimum in the Italian market reaching 0.64, it is evident that there is possibility to increase the Algeria's dates exports to those markets.

2-7. The important competitor states to Algerian dates exports in the most important markets:

2-7-1. The French market:

As shown in Table No. (10) in Annex that Tunisia is the main competitor countries to Algerian dates exports in the French market in 2006, its dates exports to France reached 13.1 thousand tons valued at 22.3 million dollars, an average price of 1,700 dollars per ton, although its production did not exceed 110 thousand tons in 2006. And Israel comes in second rank that its dates exports to the French market reached 1,200 tons valued at 4.2 million dollars at an average price of 3529 dollars per ton, although its production of dates has not exceeded 11.2 thousand tons in 2006.

The Kingdom of Saudi Arabia ranked third with the dates exports to the French market reached 8 tons valued at 10 thousand dollars at an average price of 1,250 dollars per ton, although the its production of dates has exceeded 829 thousand tons in 2006. It is clear from the foregoing that, although there is a competitive price advantage to Algerian dates in the French market, but it is observed the lack of exports to that market, which indicates that it is not related to the price, but related to marketing services as filling and packaging and other.

2-7-2. The German market:

The data table (11) in annex indicates that Tunisia is also a among the important competitor countries to exporting Algerian dates in the German market, its exports to that market 4096 tons valued at 7.8 million dollars at an average price of 1894 dollars / ton in 2002 and then it comes Iran, France, Israel, Turkey, Pakistan, Saudi Arabia, which their exports amounted to 1135, 476 410 365, 202.17 tons valued at about 1022.850, 1570.460, 99.19 thousand dollars an average price of 900.1786, 3829.1260, 490.1118 dollars per ton, while Algeria's dates exports is not exceeding to the German market 187 tons valued at 378 thousand dollars at an average price of 2021 dollars per ton in 2006.

2-8. The competitive position of Algerian dates in the most important international markets:

The Meaning of the competitivity is the ability of a country to produce goods and services that provide the needs of the international markets and help at the same time to achieve a high level of living to people of that country with continuity of this rise, competitivity measured through the competitive price indicator, and the competitive production indicator, and the value of both indicators is ranging between zero, and one true and whenever the resulting value is getting higher that means the improvement of the competitive position of the country and vice versa.
The data table (12) in Annex indicate to the increasing of the value of the competitivity price indicator of the Algerian dates in the french and german markets which the value of this indicator is one true, and 0.8, respectively .
And about the competitive production indicator for Algerian dates, it has been observed high value of this indicator in each of the French market and the German market which the value of this indicator is one true.

DISCUSSION

Despite the importance that palms sector occupied in Algeria at the both internal and external levels, the process of exporting dates is managed by not serious mechanism and characterized by a lack of competence and poor performance. Actually, it can be said that at present there is not objective system for dates trade in order to raise the value of the product and to compensate the farmer hard work, as well as ensuring the production quality and to identify as well the varieties products, this subject began to aggravate and resulted the perturbation in the dates market because of the

snaping up of wholesalers and the mediators whenever the approaching of the harvest season at the palm farms in order to monopolize the purchase the largest possible quantity of production by the lowest prices by exploiting the far away of production areas from the consumption centers and non-possession of marketing facilities for farmers and particularly the transportation, which can those traders and mediators easily impose low prices that achieve greater profits for them.

In order to improve the competitivity of the Algerian dates in the export markets, there are many ways to improve the dates competitivity in foreign markets, the most important as following:

- - Taking into account the business assets in the transaction, and invasion of new export markets in Europe, Asia, the Americas and Africa as well as to increase export capacity in the Arab markets .
- - Improve the publicity means for the Algerian dates in the international markets and the support of government to specialized organs in the external marketing to do their job.
- - The expansion in the manufacturing and packaging dates because of its importance in reducing the annual surplus production and thus to get an added value which increase the economic returns of dates through the improvement of the manufacturing means and caring the health conditions and the commercial specifications to cover the demand in the export markets .
- Encourage the investments to establish factories to produce dates in latest model, and provision of appropriate machinery to save the wet dates by cooling.
- Conducting the field and the office research to prepare the final classification of the Algerian dates and preparing standard specifications for each variety separately so that the researchers can work on rising productivity in different areas in the light of prevailing weather conditions.
- Supporting the funding and encouragement for the sector by establishment of a special fund to advancement the palm trees to cover the expenses of the agricultural works required by this sector during the year in the form of soft loans, where the applicable seasonal loan do not cover the need and affect a small part of the farmers.
- Apply the specifications to external exporting dates by precision and submitting it to strict instructions by the government agencies to ensure that the issued external exports is conformable to the special specifications of this product and guarantee thus improving the image of the Algerian dates in the external markets.
- Inserting the internationally required varieties, by planting them instead of bad varieties or adopting them from the beginning into the modern farms and test their adaptability to environmental conditions of each region.
- Reliance on modern agricultural methods, especially in the irrigation field because of its feasibility in economizing of water and reduce the spread of weeds.

And the process of improving the competitivity of dates in the external markets still requires studying the dates external markets and providing the marketing information about it (the demand volume, specification, quality, required varieties, package size, packaging, prices, competitive countries in those markets).

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Tables

Table (1): the total area and total production of dates and productivity in Algeria during the period 2000-2010

Years	Area (hectare)	index number	National production (tons)	index number	Productivity (kg)	index number
2000	101820	100	365616	100	40.83	100
2001	104390	102,52	437332	119,62	48.24	118,15
2002	120830	118,67	418427	114,44	44.65	109,36
2003	128800	126,50	492200	134,62	51.05	125,03
2004	135000	132,59	450000	123,08	45.24	110,80
2005	147906	145,26	516293	141,21	49.08	120,21
2006	154372	151,61	491188	134,35	47	115,11
2007	159871	157,01	526921	144,12	45	110,21
2008	162033	159,14	552765	151,19	44	107,76
2009	160867	157,99	600696	164,30	49.5	121,23
2010	161090	158,21	644740	176,34	50.5	123,68

Source: Agricultural Statistics series 2000-2010...

Table (2): the general trend equations of the area, and productivity, and total dates production during the period 2000-2010

Statement	The general trend equation	The correlation coefficient®	The coefficient of determination (R2)	Significant regression equation	
				Level 0.05	Level 0.01
Area	Yi=1007+6612xi	0.88	0.91	Significant	Significant
Productivity Kg / Palm	Yi=702.3+0.37xi	0.096	0.15	Not significant	Not Significant
Production (tons)	Yi=507+23297xi	0.78	0.90	Significant	Significant

Source: Data collected and calculated from the data of the ministry of Agriculture for the period 2000-2010,it was also calculated the general trend in linear form yi = a+bxi

Table(3): Evolution of the relative importance of exports of dates from the Algeria's agricultural exports during the period :2000 -2010

Years	Agricultural exports $ 1000	Rate of evolution	Exports of dates, $ 1000	Rate of evolution	The proportion of exports of dates to the total agricultural exports
2000	111000	100	14748	100	13,29
2001	154000	138,74	10441	70,80	6,78
2002	214000	192,79	16340	110,79	7,64
2003	135000	121,62	16446	111,51	12,18
2004	154000	138,74	14563	98,75	9,46
2005	164000	147,75	18493	125,39	11,28
2006	165000	148,65	20043	135,90	12,15
2007	181000	163,06	23083	156,52	12,75
2008	85000	76,58	20013	135,70	23,54
2009	273000	245,95	13698	92,88	5,02
2010	126000	113,51	16930	114,80	13,44

Source: Data were collected and calculated from the following data O.N.S, Algeria in figures, N031, 2000 Results, 2011 Edition

Table (4) : Algeria's rank among the most important dates exporters, according to the relative importance of the total global dates exports in the average period 2002-2007,

State	Exports (tons)	%Of world exports	Ranking	Exports to $ 1000	The export price in dollars per ton	Ranking	% Of world exports
World	571962	100	-	386251	675	-	100
Tunisia	46864	8,19	4	97336	2077	1	25,20
Iran	138445	24,21	2	56977	412	2	14,75
Saudi Arabia	43380	7,58	5	31623	729	5	8,19
Algeria	12931	2,26	6	18171	1405	7	4,70
Israel	7993	1,40	7	36531	4570	3	9,46
America	5485	0,96	8	14612	2664	8	3,78
Pakistan	81923	14,32	3	29392	359	6	7,61
United Arab Emirates	155805	27,24	1	31870	205	4	8,25

Source:trade Map-ITC-WTO-Market analysis section

Table (5): the evolution of dates exports in Algeria during the period 2000-2010

Years	Export quantity (tons)	Rate of evolution	Export value $ 1000	Rate of evolution	Export prices of $ / ton
2000	10783	100	14748	100	1368
2001	7850	72,80	10441	70,80	1330
2002	11022	102,22	16340	110,79	1482
2003	10198	94,57	16446	111,51	1613
2004	2585	23,97	14563	98,75	5634
2005	10863	100,74	18493	125,39	1702
2006	12328	114,33	20043	135,90	1626
2007	13356	123,86	23083	156,52	1728
2008	10055	93,25	20013	135,70	1990
2009	8945	82,95	13698	92,88	1531
2010	10393	96,38	16930	114,80	1629
Total	108378	100	184798		1967

Source: Calculated from: Agricultural Statistics Series A 2000-2010, p. 06

Table(6): Average annual export according the geographical distribution during the period 2002-2007

Years	European countries		African countries		Of American States		Asian countries		Total	
2002	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value	Quantity	Value
2003	9864	14256	636	1345	326	471	197	269	11023	16340
2004	9110	14574	299	653	598	971	190	247	10197	16446
2005	7187	12467	137	233	496	1030	314	463	8133	14563
2006	9490	15763	293	724	876	1710	204	295	10863	18493
2007	10597	17013	628	1361	114	1143	417	526	12328	20043
Total	11094	19274	706	1261	1142	2107	414	440	13356	23083
Average	57342	93347	2699	5577	3552	7432	1736	2240	65900	108968
%	9557	15558	450	929	592	1239	289	373	10983	18161
Average price	87	85.7	4.1	5.2	5.4	6.85	2.63	2.25	100	100
2002	9864	1627.9 Dollars per ton		213.8 Dollars per ton		2092.9 Dollars per ton		944.6 Dollars per ton		1653.5 Dollars per ton

Source: Collected and calculated from: Food and Agriculture Organization of the United Nations, the organization web site on the Internet.

Table (7): the relative importance of the average quantity and value of Algerian dates exports to the most important countries in the world during the period 2002-2007

Country	The average quantity of exports (tons)	The average value of exports ($ (1000)	Ratio of the quantity of exports in the continent	The proportion of the value of exports in the continent	The proportion of the quantity of exports to total exports	The proportion of the value of exports to total exports
France	8280	13355000	86.91	85.84	75.55	73.65
Belgium	551	1022000	5.8	6.57	5.03	5.64
Morocco	373	851000	82.6	89.78	3.41	4.69
Spain	470	798000	4.9	5.13	4.29	4.40
Canada	454	757000	65.7	60.4	4.14	4.18
USA	237	496000	34.3	39.6	2.16	2.74
Russia	182	214000	63.11	57.18	1.67	1.18
Sweden	78	118000	0.82	0.76	0.71	0.65
Italy	68	98000	0.72	0.63	0.62	0.54
Turkey	67	90000	23.22	24.14	0.61	0.50
Britain	35	75000	0.37	0.48	0.32	0.41
United Arab Emirates	20	37000	6.87	9.96	0.18	0.21
Guinea	14	25000	3.12	2.62	0.13	0.14
Malaysia	5	16000	1.87	4.26	0.05	0.09
Mauritania	4	15000	0.99	1.56	0.04	0.082
Netherlands	6	14000	0.07	0.09	0.06	0.077
Croatia	7	10000	0.08	0.07	0.067	0.057
Germany	4	8000	0.04	0.05	0.038	0.046

Source: Collected and calculated from: Food and Agriculture Organization of the United Nations, the organization web site on the Internet.

Table (8): the market share of Algerian dates exports, in the most important markets in 2006.

Market	Market share
France	0.45
Germany	0.25
Spain	0.22
Italy	0.11
Switzerland	0
India	0

Source: Collected and calculated from : the World Trade Center, the website of the center on the interest.

Table (9): penetration rate of the most important imported dates markets in 2006

Market	The rate of market penetration
France	**1.72**
Germany	**1.47**
India	**1**
Italy	**0.64**
Spain	**0.64**

Source: Collected and calculated from: Food and Agriculture Organization of the United Nations, the organization web site on the Internet.

Table (10) : The most important competitor countries to Algeria in exporting dates to the French market in 2006

Statement country	Exports			Production (tons)
	Quantity (tons)	Price $ / ton	Value in thousands of dollars	
	13115	1701	22304	110000
Algeria	8449	1354	11441	418427
Israel	1200	3529	4235	11200
Saudi Arabia	8	1250	10	829540
Wholesale	22772	1668	37990	1369167

Source: Collected and calculated from: the World Trade Center, the site of the center on the web.

Table (11): The most important competitor countries to Algeria in exporting dates to the German market in 2006

Statement country	Exports			Production (tons)
	Quantity (tons)	Price $ / ton	Value in thousands of dollars	
	4096	1894	7757	110000
Iran	1135	900	1022	879000
Israel	410	3829	1570	11200
France	476	1786	850	---
Turkey	365	1260	460	9400
Algeria	187	2021	378	418427
Pakistan	202	490	99	650000
Saudi Arabia	17	1118	19	829540
Wholesale	6888	1765	12155	2907567

Source: Collected and calculated from: the World Trade Center, the site of the center on the web.

Table (12): competitive indicators of Algerian dates exports in the most important markets in 2006

Market	Competitive price	Competitive production
France	1	1
Germany	0.8	1

Source: Collected and calculated from the data tables numbers (10.11).

Figures

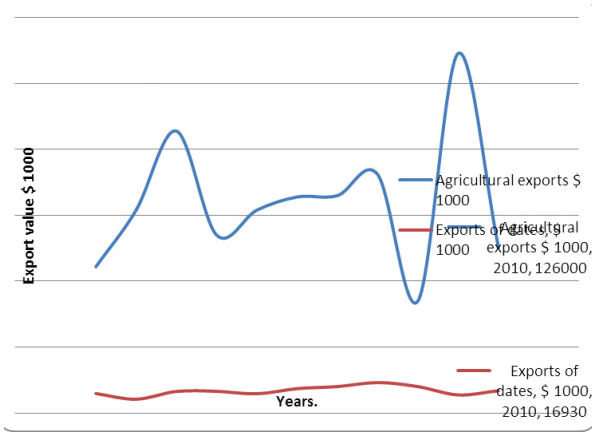
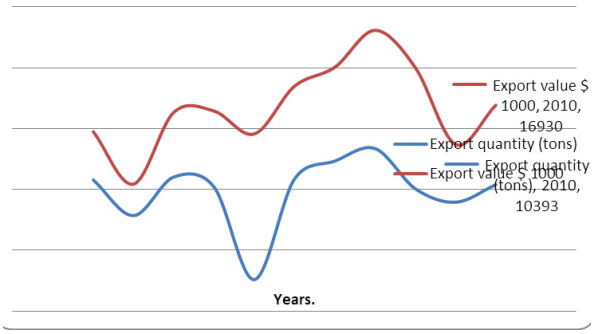


Figure (1): the evolution of the quantity and the value of dates exports of Algeria during the period 2000-2010



Figure(2): Evolution of the quantity and value of dates exports in Algeria 2000-2010

Safe methods to reduce the losses of postharvest wastage of some soft date fruits due to fungal infection during cold storage

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ABSTRACT

The principle of objectives of this study aimed to determine the losses of postharvest wastage of soft date fruits (Rutab) cvs. Zaghloul and Samani with reference to their causal organisms. Posssibilities to control these disease by safe methods such as green algae and propolis extracts. The main causal organisms of postharvest fruits rots of two tested cvs. of date were *Botrytis cinerea*, penicillum *expensum* and *Rhizopus nigricans* during cold storage periods in two seasons (2010 and 2011). Fruits of the two tested cvs. were stored under cold storage (5 ±1 oC). Fruits of each tested were coating with green algae or propolis extracts, the results obtained after 30 and 60 days from treatments under cold storage condition, indicated that the most effective concentration was 100% to green algae and 30% to propolis extracts in two seasons, which reduced percentage of total wastage than control after 30,60 days on cvs. Zaghloul and Samani to (27.4, 35.2%), (28.5, 39.9 %) and (19.1,30.4%), (27.6,46.1%) in the first season and (23.1,33.0%), (25.1, 46.8%) and (17.4,28.9%), (37.6,48.1%) in the second season respectively, coating fruits of two tested cvs. with extracts of green algae (100%) or propolis (30%) caused greater reduction in weight loss, fruits shatter and decay percentages than which obtained at lower concentrations (25, 50%) to green algae or (10,20%) to propolis when fruits were stored 30 and 60 days under cold storage in two seasons.

Key words: Cold storage- Fungi - Green algae- Propolis extract - Soft Date Fruits.

INTRODUCTION

Date palm fruits, particularly those of Zaghloul and Samani cultivars are one to the most luxurious fruits and have high nutritive value (EL- Badawy, 2001).

No doubt that the processes of handling and storage of date fruits for local market and export are as important as fruits production and fruit yield. The extension of marketing period using postharvest treatments is of vital interest. Moreover, as a result of increasing the supply of date fruits, there is a desperate need for studying how the marketing period could be extended and how to reduce loss of fruits and to supply date fruits frequently and over a long period of time. Consequently, storage of date fruits is necessary to regulate the supply of date fruits according to marketing need over a long period of time.

Temperature has a direct effect on the respiration rates of fruits and on the decay percentage caused by the activity of organisms. The respiration rate is an index of the rate at which the fruits is using up its stored reserves of sugars and other metabolites and consequently, an index of the loss in shelf life.

The chemical reactions associated with respiration, results in the production of heat. The amount of generated heat varies with the commodity and with its temperature.

In general, the respiration rate increase two to four times for each 10 oC increase in temperature, and cold storage is required to reduce heat generation and decay percentage and proloning storage life (Abd El- Moniem, Eman and Magda Abd El- Migeed, 2006, Abd El-Migeed and Fatouh, 2007, and Raweewon 2008,).

Recently a great attention has been paid by many investigators all over the world concerning the growing need to develop alternative approaches for controlling postharvest decay.

Coating fruits with green algae decreased postharvest decay of tomato, strawberries and grapes resulting from fungal infection (Abd El- Moniem Eman, *et al.,* 2005) and has been widely used in medicine, agricultural production, (Benhamou *et al.,* 1998; El-Bardai, *et al.,* 2001, El- Gamal, Manal, 2006 and Calvo, 2007).

Recently, coating application of biochemical organic substances, which supply both macro and micronutrients, is of increased demand because they have the advantage that they are safe to human and environment.

Fresh water green microalgae contains high percentage of macro and micronutrients bounded in their major biochemical constituents such as amino acid, carbohydrates and proteins (El- Fouly *et al.,* 1992 and El – Fouly and Shaaban 1999).

Recent health concerns over pesticide contamination of food, public awarence towards chemical residues in the food chain. National Academy of Science report (Anonymous, 1987b) on pesticides residues indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides. All those factors together have generated an urgent need for the development of safer alternative technologies.

Propolis or bee glue, is a brownish resinous material collected by worker bees from the leaf buds of numerous tree species. The Term propolis – derives from the Greek pro (for in front of at the entrance to) and polis (community' or city) and means a substance in defense of the hive (Jin, 2002).

The major propolis compound are resins composed of flavonoids and phenolic acids, or their esters, which often form up to 50% of all ingredients, the antioxidant. Antimicrobial and antifungal activities of propolis offer scope for applications in food technology. One special advantage is that unlike some conventional preventatives, the residues of propolis seem to have a generally beneficial effect on human health (Krell, 1996).

Propolis has antioxidant, antimicrobial and antifungal activities where diseases are the principal factor limiting manog storage life (Abd EL-migeed and Fatouh, 2007).

Propolis has advantages of coating material that impact on storage life. Propolis reduces enzyme activity (Jin., 2002). This may impact on reducing changes during storage of mango fruits.

The future post- harvest research should aim to provide methods to control ripening avoid or minimize physiological disorders and to provide maximum fruit quality to the consumer.

The present work aims at evaluating the efficacy of the water extract of cell green algae (chlorella vulgaris) or proporis extract as coating treatment to recue the losses resulted from postharvest of date fruits, (cvs. Zaghloul and Samani). Possibilities to reducing this wastage by water extract of green algae or propolis extracts and testing it in cold storage against the most important postharvest decay pathogens of date fruits was also investigated.

MATERIASL AND METHODS

I- Isolation and identification of the causal pathogens:

Date fruits (cvs. Zaghloul and Samani) were stored 30 and 60 days at (5 ±1 oC). Each treatments consisted of three replicates 3Kg ± 250g each. Fruits were examined during storage at 30 and 60 days. The ones showed rotten symptoms, were used for isolation the causal which caused date rots according to (Waller, 1981) and identified according to (Barnett & Hunter, 1987).

II- Post harvest treatment of date fruits, with algae fresh cells during storage:

1- Preparation of algae extract:

A fresh slurry of the microalga Chlorella vulgaris (contains about 10% water) was washed with distilled water, reconcentrated by centrifugation and freezed and then remelted at room temperature. The melted slurry was then centrifuged at 5000 rpm to obtain a clear cell sap. Major components and nutrient content of the algae extract is shown in Table (1).

2- Coating date fruits with algae cell extract:

Fresh of date (cvs. Zaghloul and Samani) apparently free of physical damage and diseases were utilized to be coated with algae cell extract. Fruits of the two tested cultivars were dipped in 25,50, or 100% green algae cell extract. Control were dipped in sterilized water. Tested fruits were air dried for 2 hour and packed in carton boxes (45 x 35x 10cm) and directly stored at (5 ±1 oC). Each treatment included replicates, 3Kg ± 250g each.

3- Ethanol extracted propolis (EEP)

Preparation for extraction: The propolis was prepared by removing coarse debris and excessive wax then be broken into small pieces. Propolis concentrations at rates 10, 20 and 30% were prepared by weighing propolis at rates 100, 200 and 300g then each rate of propolis poured with 900, 800 and 700g ethyl alcohol respectively into 1L clean, dark colored bottle which can be tightly closed then be shaken briefly. Shaking was repeated twice a day; the mixture was left in a warm dark place. After one weeks the liquid was filtered through a paper filters. Twice finally, the filtrate was kept in a cool dark place (Krell., 1996).

II-Determine of post harvest treatments of date fruits with algae or propolis extracts under cold storage periods:

Percentage of decay (%): The detection of decay was carried out at 30 and 60 days under cold storage. Fruit date showed symptoms of decay were detached and the percentage of decay was calculated by weight as follows:

$$\text{Decay}\,\% = \frac{\text{weight of decayed date fruits (g.)}}{\text{initial weight of date fruits (g.)}} \times 100$$

Weight loss (%): Was calculated by weighting of sample date fruits each 30 and 60 days during cold storage the initial weight of date fruits were recorded at zero- time:

The following formula was used to determine the percentage of weight loss.

$$\text{Weight loss}\,\% = \frac{\text{Initial weight} - \text{Weight of Sampling date}}{\text{initial weight of the date fruits (g.)}} \times 100$$

Shattering percentage: The value of shatter fruits was determined as follow:

$$\text{Shattering}\,(\%) = \frac{\text{weight of shattered fruits}}{\text{initial weight of date fruits}} \times 100$$

Wastage percentage: The value of wastage (%) was determined according to (Wassel, 1985) as fellow:

Wastage (%) = Decay % + Weight loss % + Shattering %.

Reduction percentage: The reduction in wastage fruits was calculated as compared to wastage of control:

$$\text{Reduction of wastage}\,(\%) = \frac{\text{Total wastage of treatment} - \text{Control}}{\text{Control}} \times 100$$

Statistical analysis:

Data obtained were statistically analyzed when necessary using L.S.D. procedure outlined (Snedecor and Cochran, 1982) and using the standard procedure for split designs mentioned by (Snedecor and Cochran 1967).

RESULTS

1-Frequency and identification of isolated fungi (%) causing rot to date fruits:

Date fruits of Zahgloul and Samani were harvested at full coloured stage (Khlal) were stored at (5 ±1 oC) and examined at 30 and 60 days during storage for rot causal organisms. Date fruits, which showed rot symptoms, were subjected to isolating and culturing the associated fungi. Frequency of various isolated fungi during cold storage period are presented in table (3). Data in table 3 show that in cv. Zaghloul the frequency of *Botrytis* recorded (37.6, 27.4%) after 30 and 60 days, while the frequency of *Penicillum* recorded (21.1, 29.5%) in the first season. Mean while, in the second season recorded (32.7, 28.6%) and (19.6, 24.3%). in cv. Samani, recorded (36.3, 36.5%), for *Botrytis* and (22.7, 24.9) for *Penicillum* in the first reason, while in the second for *Botrytis* receded (32.6, 37.5) and (22.9, 26.6%) for *Penicillum*. Results indicate that, the percentage of frequency of the fungi, *Botrytis*,*Penicillum* and *Rhizopus* increased as the storage periods increased.

The isolates that exhibited the highest percentage of frequency were identified as *Botrytis cinerea*, *Penicillum expensum* and *Rhizopus nigricans*

2- Fruit decay percentage

Data in Table (4, 5) show that decay percentage is storage temperature dependent. In other words, the lower storage temperature the longer is the storage period. The obtained results emphasize these words, hence the storage period under cold storage at 5oC was extended up to 60 days in two seasons. Moreover, it is clear that Samani fruits proved to be more tolerant to decay agents during the storage period. Besides, Zaghloul fruits recorded nearly similar values of decay percentage throughout the storage period. As for the effect of fruit treatment with algae or propolis extracts on decay percentage, it is obvious that two treatments succeeded in reducing decay percentage as compared with the control. On the contrary, untreated fruits (control) of the two studied cultivars recorded comparatively higher decay percentage.

3- Fruit weight loss percentage:

Furthermore tables (4,5) demonstrates that the interaction between the cultivar and green algae or propolis extracts treatments induced a pronounced effect on weight loss percentage of date fruits. Briefly, Samani fruits treated with

100% algae or 30% propolis showed statistically similar and the lowest weight loss percentage during the storage period 30 and 60 days in two seasons, followed ascendingly by the analogous ones of Zaghloul cv. treated with 100% algae or 30% propolis extracts. On the contrary, untreated fruits (control) of Zaghloul and Samani cvs. recorded the highest values of fruit weight loss. Other studied combinations gave in between values in this respect.

4- Fruits shattering percentage:

Data of presented in Table (4, 5) indicate that two tested cvs. fruits shattering percentage increased by progress in cold storage period to 60 days in two seasons. However, fruits treated with 100% algae or 30% propolis extracts had lower fruits shattering percentage (45, 38%) and (42, 33%) for Zaghloul and Samani in the first season and (47, 30%), (40, 35%) in the second season after 60 from cold storage.

5- Total wastage and reduction than control percentage:

Data presented in Table (4, 5) show that the highest percentage of total wastage and reduction than control were determined after 60 days, from cold storage at (5 ±1 oC). Algae 100% or propolis extracts 30% resulted in the highest reduction in total wastage and reduction than control. For Zaghloul cv. fruits treated with algae 100% or propolis 30% recorded (78.2%, 65.8%) total wastage and reduction than control recorded (28.5%, 39.9%) in the first season and (81.7%, 58.0%) total wastage and reduction than control recorded (25.1 %, 46.8%) in the second season. For Samani cv. recorded (70.7, 52.6%) total wastage and reduction than control recorded (27.6, 46.1%) in the first season and (64.1, 53.3%) total wastage and reduction than control recorded (37.6, 48.1%) in the second season.

6- Shelf life:

The effect of cultivar namely Zaghloul and Samani, fruits treatment with algae 100% or propolis extracts 30% on shalf life of date fruits stored at (5 ±1 oC) is illustreated in Table (6). It is obvious that increasing storage period at room temperature resulted in increasing decay percentage at room temperature and shelf life was decreased. Moreover the two tested treatments succeeded in enhancing shelf life of date fruits stored at (5 ±1 oC).

Zaghloul and Samani cultivars treated with 100% algae or 30% propolis extracts exerted equally and highly positive effect in this respect. Besides, untreated (control) and separated fruits of Zaghloul and Samani gave the least positive effect in this sphere the remaining interactions came in between in this concern.

DISCUSSION

Date fruits are one of the largest cultivated fruit crops in several countries. Fruits are mostly subjected to infection during handling, transportation and storage, which causes high losses. Study the problem was to reach safe alternative for preservation against postharvest decay and prolonging storage period of date fruits.

Fruits of Zaghlgul and Samani cultivars were stored at cold storage (5 ±1 oC) for 60 days. Samples were taken every 30 days for evolution. The experiment was repeated for two seasons (2010 and 2011).

Botrytis ceinerea, *Penicillium expensium* and *Rhizopus nigricans* were the most frequent fungi on three tested cultivars during storage in the two seasons. *Botrytis* rot is known to be the most widespred and major cause of deterioration of date fruits in cold storage (Hoa *et al.,* 2002 and Tripathi and Dubey, 2004). This of ungns is the main decay problem all over the world in date fruits exposed in the field to high humidity.

As for decay in date fruits, weight loss, shattering and total wastage estimated during storage, four those factors increased as the time of storage was increased.

The role of cold storage reducing decay percentage could be explained by the fact that the chemical reactions associated with respiration results in the production of heat. Microbial organisms also are more active at high than low temperature. Therefore, cold storage is required to reduce this generation of heat and fruit decay. The results of cold storage and related discussions are in harmony with the finding of on Samani and Zaghloul date fruits of (El Badawy, 2001) on date palm fruits (Mehaisen, 2005 a and b) in guava and pear.

Green algae or propolis extracts could be used as a substitute of fungicides to inhibit postharvest decay. Green algae or propolis extracts coating decreased postharvest decay of several fruits (Abd El – moniem, Eman *et al.,* 2005) on grapes and (Abd El – Migeed and Fatouh 2007) on mango fruits. These findings are confirming with the obtained results which approved that green algae or propolis extracts at high concentration were effective in preventing postharvest decay of grape fruits. The effects appear to be related the fungistatic property of the coat as indicated by (Abd El-Moniem, Eman *et al.,* 2008 and Soliman, *et al.,* 2009).

It could be suggested that green algae or propolis extracts might be safty used commercially as fruit coating to control postharvest disease and for prolonging the shelf life of sensitive fruits.

The role of green algae and propolis extracts in reducing the decay percentage may be due to the fact that calcium appears

to have an important regulating effect in the metabolism of date fruits. Metabolic disorders such as bitter pit, cork spot, internal breakdown and water core are all severely reduced if calcium is present in sufficiently high quantities in fruit. This suggests that calcium may regulate respiration and perhaps other metabolic processes in the mature fruit.

The results reported that coating of green algae significantly reduced the respiration rate, ethylene production and interval O2 level of Awis mango. (Abd El – Migeed and Fatouh 2007) reported that treatment with propolis coat were effective in decreasing weight loss and reducing respiration rate. Mean wile, results indicate that all propolis extract concentrations significantly decreased the disease incidence of mango fruits, and promising treatment to prolong shelf life, preserve fruit quality and reducing disease incidence significantly.

The weight loss is mainly a result of water loss from the fruit tissues and partially of the respiration process. The higher storage temperature, the higher are the respiration rate and weight loss (Mehaisen, 2005 a and b). He mentioned that the higher the air temperature, the more is water loss because of its capacity to evaporate water (Diga et al, 2000) on salustina orange fruits (El Badawy, 2001) on date palm fruits and (Mehaisen 2005b) on pear fruits.

They mentioned that there was reversible relationship between the storage temperature and weight loss. Moreover, the role of green algae or propolis extracts treatments on reducing weight losse was reported earlier by (Daood, 1995) on Zaghloul dates, (Bhartiya *et al.,* 1998) on apple fruits, (Farooq *et al.,* 1999 and Mehaisen 2005b) on pear fruits. They demonstrated that the physiological weight loss was generally lower in green algae treated fruits.

Use of algae cell or propolis extracts decreased the percentage of total wastage as well as precnetage of decay. Highly significant reverse correlation were found between the increased concentration of extract and percentage of total wastage.

Nutrients present in the cell of green algae or propolis extracts, which mostly are in organic from can be directly involved in the metabolism. Mean while, the amino acids derived from proteolysis can work as chelating agents, facilitating the penetration of elements through fruits (El Fouly, *et al.,* 1992). Amino acids can also migrate to fruits and play a role as phytosiderphores, facilitating the absorption of micronutrients through the fruits (Shaaban and Mobarak 2000). Moreover, algae extract as a natural plant cell sap contains certain amounts of hormones, enzymes and vitamins that may improve nutrient assimilation.

As for shelf life, the results of storage temperature go in line with finding of Zhang *et al.,* (2000) on Litchi. Besides, the obtained results of fruits treatment with green algae or propolis extracts are in harmony with those mentioned earlier by (Freire and Chitarra 1999) on mango, (Mehaisen 2005a&b) on pear and (Abd El Mgeed& Fatouh 2007) on mango fruits.

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Tables

Table 1: Major chemical composition and elemental contents of algae Chlorella vulgaris cell extract. (El-Fouly *et al.,* 1992).

General composition			Element content	
Protein (%)		44	**Macro-elements (%)**	
Fats (%)		6	N	7.1
Carbohydrate (%)		7.3	P	0.66
Amino acid composition (g/100g protein)*		12	K	2.15
Arginine	6.9	8	Mg	0.34
Histidine	2.0		Ca	0.18
Isoleucine	3.2		Na	0.04
Lucien	9.5			
Lysine	6.4	48.6	**Micro-elements (ppm)**	
Methionine	1.3	6	Fe	245
Phyenylalanine	5.5		Mn	131.2
Theronine	5.3		Zn	111.5
Tryptophan	1.5		Cu	28
Valine	7.0			

The treatment was carried out at three replicates.
Control: distilled water
T1: 25% (v/v) algae cell extract in distilled water.
T2: 50% (v/v) algae cell extract in distilled water.
T3: 100% (v/v) algae cell extract.

Table 2: The major compounds of propolis .

Class of components	Group of components
Resins	45 to 55% Flavonoids, Phenolic acids and esters 25 to 35%
Wax and fatty acids	Most are usually from bee wax, but may are of plant origin
Essential oils	10% volatiles
Pollen	5% Proteins probably from pollen: free amino acids.
Other organics and minerals	5% 14 trace minerals of which Fe and Zn are most common ketones, lactones, quinines, steroids, benzoic acid and esters, vitamins (only B) and sugars.

Table 3: Frequency of various isolated fungi(%)causing rot to date fruits cvs. Zaghloul and Samani under cold storage conditions (5 ±1 oC)

Isolates Fungal group	1st Season (2010)							
	cv. Zaghloul				cv. Samani			
	30 days		60 days		30 days		60 days	
	No. of date fruits	% isolation	No. of date fruits	% isolation	No. of date fruits	% isolation	No. of date fruits	% isolation
Botrytis cinerea	320	37.6	195	27.4	205	36.3	183	36.5
Penicillum expensium	180	21.1	210	29.5	128	22.7	125	24.9
Rhizopus nigricaus	135	15.8	122	17.1	105	18.6	98	19.5
Fusarium sp.	90	10.6	100	14.1	65	11.4	60	11.9
Alternaria alternata	55	6.5	-	0.0	-	0.0	-	0.0
Aspergillus sp.	50	5.9	85	11.9	42	7.4	36	7.2
Others	22	2.6	-	0.0	20	3.6	-	0.0
Total	852	100	712	100	565	100	502	100
2nd Season (2011)								
Botrytis cinerea	272	32.7	125	28.6	138	32.6	120	37.6
Penicillum expensium	163	19.6	106	24.3	97	22.9	85	26.6
Rhizopus nigricaus	128	15.4	73	16.7	85	20.1	66	20.7
Fusarium sp.	96	11.5	65	14.9	50	11.8	30	9.5
Alternaria alternata	78	9.4	48	10.9	-	0.0	-	0.0
Aspergillus sp.	63	7.6	20	4.6	35	8.3	18	5.6
Others	32	3.8	-	0.0	18	4.3	-	0.0
Total	832	100	437	100	423	100	319	100

Table 4: Effect of green microalgae and propolis extracts as fruit coating (%) on total wastage and reduction than control percentage of date fruits (cv. Zaghloul) stored at cold storage (5 ±1 oC)and (90-95 R.H.) after 60 days from cold storage.

1st Season (2010)											
Cold storage periods (5 ±1 oC)											
		30 days					60 days				
Treatments	Con.%	Decay %	Weight loss %	Fruit shatter %	Total Wastage %	Reduction than Control%	Decay %	Weight loss %	Fruit shatter %	Total Wastage %	Reduction than Control%
Control (untreated)	-	8.7	6.4	25.0	40.1	-	34.6	9.8	65.0	109.4	-
Green algae extract	25	8.5	5.2	23.0	36.7	8.5	33.0	10.0	53.0	96.0	12.3
	50	6.9	5.0	21.0	32.9	18.0	30.3	8.5	50.0	88.8	18.8
	100	5.5	4.6	19.0	29.1	27.4	25.2	8.0	45.0	78.2	28.5
Propolis extract	10	6.0	5.1	22.0	33.1	17.5	26.0	8.1	45.0	79.1	27.7
	20	4.5	4.3	20.0	28.8	28.2	21.5	7.8	40.0	69.3	36.7
	30	4.0	4.0	18.0	26.0	35.2	20.6	7.2	38.0	65.2	39.9

L.S.D. at 5% for : Treatments (T)= 1.6 Storage (S) = 2.1 (T) x (S) = 4.3

2nd Season (2011)											
Control(untreated)	-	7.8	5.6	26.0	39.4	-	35.8	10.3	63.0	109.1	-
Green algae extract	25	7.7	5.0	24.0	36.7	6.9	32.0	9.5	55.0	96.5	11.6
	50	5.9	4.9	20.0	30.8	21.8	27.0	9.1	50.0	86.1	21.1
	100	5.6	4.7	20.0	30.3	23.1	25.7	9.0	47.0	81.7	25.1
Propolis extract	10	6.1	4.6	22.0	32.7	17.0	26.0	10.0	42.0	78.0	28.5
	20	5.5	4.1	21.0	30.6	22.3	22.3	8.6	35.0	65.9	39.6
	30	4.0	3.4	19.0	26.4	33.0	20.0	8.0	30.0	58.0	46.8

L.S.D. at 5% for : Treatments (T)= 1.4 Storage (S) = 2.6 (T) x (S) = 3.8

Table 5: Effect of green microalgae and propolis extracts as fruit coating (%) on total wastage and reduction than control percentage of date fruits (cv. Samani) stored at cold storage (5 ±1 oC) and (90-95 R.H.) after 60 days from cold storage. w

1st Season (2010)											
Cold storage periods (5 ±1 oC)											
		30 days					60 days				
Treatments	Con.%	Decay %	Weight loss %	Fruit shatter %	Total Wastage %	Reduction than Control%	Decay %	Weight loss %	Fruit shatter %	Total Wastage %	Reduction than Control%
Control (untreated)	-	4.3	4.5	32.0	40.8	-	29.0	8.6	60.0	97.6	-
Green algae extract	25	4.0	4.3	31.0	39.3	3.7	27.1	8.5	55.0	90.6	7.2
	50	2.7	4.0	30.0	36.7	10.0	25.0	8.0	49.0	82.0	16.0
	100	2.3	3.7	27.0	33.0	19.1	21.5	7.2	42.0	70.7	27.6
Propolis extract	10	2.5	3.8	31.0	37.3	8.6	20.0	7.3	45.0	72.3	25.9
	20	1.8	3.2	28.0	33.0	19.1	15.7	7.1	40.0	62.8	35.7
	30	1.5	2.9	24.0	28.4	30.4	12.6	7.0	33.0	52.6	46.1

L.S.D. at 5% for : Treatments (T)= 2.7 Storage (S) = 3.1 (T) x (S) = 6.2

2nd Season (2011)											
Control(untreated)	-	5.5	3.7	34.0	43.2	-	27.0	8.7	67.0	102.7	-
Green algae extract	25	4.9	3.5	33.0	41.4	4.2	25.3	7.6	63.0	95.9	6.6
	50	4.7	3.0	30.0	37.7	12.7	20.0	7.3	50.0	77.3	24.7
	100	4.1	2.6	29.0	35.7	17.4	17.1	7.0	40.0	64.1	37.6
Propolis extract	10	4.0	3.1	30.0	37.1	14.1	18.1	7.1	40.0	65.2	36.5
	20	3.3	2.3	28.0	33.6	22.2	16.2	6.5	38.0	60.7	40.9
	30	2.6	2.1	26.0	30.7	28.9	12.0	6.3	35.0	53.3	48.1

L.S.D. at 5% for : Treatments (T)= 2.2 Storage (S) = 4.4 (T) x (S) = 5.8

Table 6: Effect of green microalgae and propolis extracts as coating treatments on shelf life date fruits stored at cold storage (5 ±1 oC)and (90-95 R.H) (after 60 days from cold storage).

Treatments	Conc. %	cv. Zaghloul			cv. Samani		
		Storage on shelf life (days) decay %					
		2	4	6	2	4	6
1st Season (2010)							
Control(untreated)	-	40	55	90	30	45	70
Green algae extract	25	40	50	80	30	40	65
	50	30	40	50	20	30	40
	100	25	30	45	20	30	40
Propolis extract	10	20	30	45	20	30	35
	20	20	30	40	20	25	30
	30	15	20	40	10	25	30
L.S.D. at 5% for:		Treatment (T)= 3.5 Cultivars (C) =1.5 (T) x(C) =4.8					
2nd Season (2011)							
Control(untreated)	-	40	50	75	30	40	65
Green algae extract	25	40	55	75	30	40	60
	50	20	50	60	20	30	40
	100	20	35	40	20	30	40
Propolis extract	10	20	30	45	20	25	35
	20	15	40	45	15	20	30
	30	10	25	35	15	20	30
L.S.D. at 5% for:		Treatment (T)= 4.1 Cultivars (C) =1.8 (T) x (C) =5.2					

Improving fruit quality and prolonging the marketable periods to reduced the infection with insect and fungi of some dry date fruits

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ABSTRACT

This investigation was carried out during two successive seasons (2010/2011 and 2011/2012) on two dry date cultivars (Sackoti and Bartamoda), Fruits selected for the study were nearly uniform and free of visual defects and divided into six groups. Fruits of the first group left without treatment, while, the second was treated with heat (40oC for 72 hours), the third group was treated with methyl bromide (MB) fumigation, the fourth, the fifth and the sixth groups were irradiated with doses of gamma rays at levels 0.25, 0.50 and 1.00KGy respectively. The six groups were stored on shelves at the room conditions with temperature range of 20-30oC and relative humidity 60-70% for 10 months. Fruits were evaluated every two months, for infection, weight loss, moisture content, total, reducing sugars and tannins percentage. Results in both seasons showed that storage duration is directly proportion to the increase in insect (*Oryzaephillus surinamensis*) & fungi (*Penicillum expensium*, *Aspergillus* niger and *Rhizoups nigricans*) and weight loss. While, moisture content, total and reducing sugars percentage were decreased by increasing the storage period in all treatments for different date fruits under investigation. Among treatments, methyl bromide fumigation and irradiation with dose of rays at the highest level were a favorable treatment for minimizing weight loss in both cvs., while heat treatment was superior keeping the Marketable fruit properties with regard to weight loss, total reducing sugars of (cv.Bartamoda) date fruits and moisture content of (cv. Sackoti) and insect & fungal infection in all date fruits under study. However, methyl bromide treatment resulted in decreasing reducing sugars (%) in (cvs. Sackoti and Bartamoda) fruits. Generally, it could be concluded that fruit treated with methyl bromide or irradiation dose (1.00 KGy) stored for 10 months is the promising treatment to prolong shelf life, preserve fruit quality and reducing infection incidence significantly.

Key words: (cvs. Sackoti and Bartamoda) date fruits - Fungi & Insect - Heat - Irradiation - Methyl bromide.

INTRODUCTION

The date palm (*Phoenix dactylifera*, L.) is one of the oldest cultivated fruits in the world. Dates are one of the richest source of nutrients mainly sugars, vitamins, proteins, sodium, iron, magnesium, and other minerals. Date fruits may be eaten fresh or utilized industrially in the production of syrup, liquid sugars, vinegar, ethyl alcohol, yeast, pastry and animal feeds. Date fruits are classified according to their moisture content into soft date exceeding 30% moisture, semi- dry dates with moisture content ranging between 20-30%, and dry dates with less than 20% moisture content (El- Badawy, 2001). Semi-dry dates are concentrated in Sharkia and Giza Governorates,

while dry dates dominate in Upper Egypt. Cultivated date palms in Egypt (soft, semi- dry, and dry) reached 7951247 palms which produced 746039 tons annually according to the Latest Agricultural Statistics 2011 (Agricultural Economic Reports (2011), Ministry of Agriculture, Dokki, Giza, Egypt).

Recently, the Ministry of Agriculture has demonstrated great interest in increasing agricultural products as well as to reduce post harvest losses. Reports showed that, developing countries suffer major post harvest losses up to 40% of their agricultural output. Unfortunately, date fruits are easily attached by different organisms i.e. insects and microbial flora during storage which causes rotting and spoilage of the fruit. Currently dates are preserved by many methods such as low temperature, steam treatment, pasteurization drying, fumigation, low temperature storage and packing in vacuum or inert gas. Higher temperature (more than 55oC) induced an adverse effect on the colour and flavour, whiel drying gives an uneven rough, fibrous or rubbery texture. Methyl bromide is the main fumigant used for insect disinfestation. However, its application causes serious problems i.e. residue accumulation and in complete kill of some stages of the insect (Atwa, Marwa 1999). The other used methods of preservation are too expensive and need special facilities. Consequently, new methods of date disinfestations have been developed. The most successful method in this respect is irradiation.

Irradiation treatment is very effective in eradicating insect infestation in packed dried dates, reducing of microbial contamination, and prolonging the shelf life of fresh dates. Different United Nation Agencies mainly World Health Organization (WHO), food and Agriculture Organization (FAO), and International Atomic Energy Agency (IAEA) have jointly confirmed that irradiation is unconditionally safe for a wide range of foods when the absorbed dose do not exceed 10KGy. Meanwhile, heat plus irradiation, greatly reduce the negative effects of irradiation (Romani, 2004).

Thus, the ultimate goal of this investigation is to find out the best alternative method for maintaining fruit quality and prolonging the marketable period of date fruits. Meanwhile, the selection of the most suitable dose of irradiation to valuable in reducing fruit loss and in turn to increase the income of date producers.

The aim of this study is to find out the best alternative method for improving fruit quality as well as prolonging the marketable period of date fruits.

MATERIALS AND METHODS

The present study was conducted on two dry date cultivars (Sackoti and Bartamoda) during two successive seasons (2010/2011) and (2011/2012).

Dry dates were brought from Aswan Governorate. One hundred and sixty two kilograms of fruits, from each date cultivar fifty four Kg. in each season were collected to be used for different experiments. Fruits selected for the study were nearly uniform and free of visual effects, the fruits of two tested cultivars were packed in carton boxes (30x20x20cm) previously treated with Cifadex as fungicide each box 3 kg and divided into six groups each groups contain of three tested cultivars (semidry and dray date)as follows:

- Fruits of the first group were left without any treatment (control)
- Fruits of the second group were treated with heat treatment in the oven at 40oC for 72 hours during two seasons was used for both semidry and dry date fruits.
- Fruits of third group were treated with methyl bromide (MB) fumigation treatment.
- Fruit of fourth irradiated in a cobalt – 60 source with dose of gamma rays at level 0.25, 0.50 and 1.00KGy.

Irradiation with a cobalt- 60 source in National Center for Radiation Research and Technology (NCRRT), in Cairo.

All packed samples of both dry date fruits were stored on shelves at the room temperature conditions with temperature rang of 20-30oC and relative humidity 60-70% for 10 months. Meanwhile, samples were taken every two months interval from zero time up to the end of storage period and subjected to the following measurements.

A- Physical characters:

1- Insect and fungi infestation %: The detection of fruits infection was carried out at two months intervals from zero time (start) up to the end of storage period. From each treatment fruit were examined and checked visually from both outside and inside to show any infection with insect stages as (eggs, larvae, adults) or fungi. The infested fruits were discarded and percentage of infection was calculated by weight as follows:

$$\text{Infection}\,\% = \frac{\text{weight of infection of date fruits (g.)}}{\text{initial weight of date fruits (g.)}} \times 100$$

a- Identification of insect: At the end of storage period for 10 months the insect has been identified by plant protection Dept. N.R.C..

b- Isolation and identification the causal pathogens: At the end of storage period for 10 months the ones showed rotten symptoms from inside and outside of fruit, were used for isolation the pathogen which caused date infection according to (Waller, 1981) and identified according to (Barnett & Hunter, 1987) after isolated on PDA media, incubation for 10-15 days on 25 oC.

2- Fruit weight loss%: was calculated by weighting the same each two months during storage, the initial weight of date fruits were recorded at zero time:

The following formula was used to determined the percentage of weight loss:

$$\text{Weight loss \%} = \frac{\text{Initial weight - weigth of smapling date}}{\text{initial weight of date fruits (g.)}} \times 100$$

3- Moisture content %: Fruits were cleaned and the seeds were removed, then flesh was minced and dried at 60-65oC for 48 hours according to (Abd El- Rahman, 1974) method.

B- Chemical analysis:

1- Total and reducing sugars percentage

Total and reducing sugars were determined using the colorimetric method as recommended by (A.O.A.C., 1990) method.

2- Tannins percentage:

Tannins were determined according to the (A.O.A.C., 1990) method.

Statistical analysis:

All obtained data of this study were subjected to the analysis of variance using the general linear model procedure of SAS, (1989), where appropriate treatment means were separated using Duncan's multiple rang test, (Duncan, 1955). Moreover, percentage were transferred to angles before statistical analysis.

RESULTS AND DISCUSSION

Physical characteristics:

1- Insect and fungi infestation %:

a- Identification of insect: The insect identified as (*Oryzaephillus surinamensis*) adults.

b- Isolation and identification the causal pathogens:

Data in Table (1), shows that seven genera of fungus were isolated from outside date fruits. Fungi were purified and identified as *Penicillum expensium* (Link), *Aspergillus* niger (Vantighem) and *Rhizoups nigricans* (Ehrenberg), *Stemphyllium* sp. (Waller), Mucor sp. (Micheli), *Geotrichum* sp. (Link) and *Trichothecium* sp. (Link).

Penicillum was the most frequently isolated fungus followed by *Aspergillus* and *Rhizoups* respectively, while the other isolated fungi were found at low frequencies.

2- Fruits infection %:

Data presented in Table (2), the percentage of Sackoti infestation date fruits increased with increasing storage period. Meanwhile, percentage of fruits free of infestation increased significantly by increasing the doses of irradiation. Methyl bromide treatment was promising in reducing the percentage of infestation followed by irradiation and heat treatments as compared with the control.

Regarding the effect of heat, methyl bromide and irradiation treatments on the percentage of Bartamoda infection date fruits, table (2) clarify that prolonged storage duration adversely affect percentage of fruits free of infestation. Concerning irradiation and methyl bromide treatments, it is clear that all previous treatments decreased the percentage of infection fruits. Comparing different doses of irradiation it is quite evident that the highest doses were more superior in this respect. However, (0.50KGy) dose was statistically more or less similar, but still higher in their effect than the lower dose (0.25KGy). In general, methyl bromide and irradiation treatments decreased in the percentage of fruits infected followed by heat treatments as compared with the control.

These results confirmed findings obtained by (Ahmed, et.al, 1985, 1987) indicated the importance of treatment with Gamma ray and methyl bromide for killing all (*Oryzaephillus surinamensis*) adults and ensures full sterility and complete inhibition of reproduction of this infection (Gagnon & Lacroix, 1993). On mangoes fruits, (Hassouna et. al, 1994) on date palm fruits, (El- Salhy, Fatemah, 1998) on Sackoti and Bartamoda date fruits (El- Badwy, 2001) on date palm fruits and (Thomas, et. al, 2005) on grapes.

3- Fruits weight loss percentage:

Data in Table (3) clarify that weight loss percentage of Sackoti date fruits is directly proportional and coincided with the increase in storage duration in all treatments. However, the highest irradiation dose (1.00KGy) induced the lowest significant weight loss followed by methyl bromide and the lowest doses of irradiation (0.25 and 0.50KGy) as compared with the control in a descending order. In the second season, show that weight loss was increased by increasing period in all irradiation and methyl bromide treatments. In general, irradiation treatments succeded in decreasing weight loss bollowed by methyl bromide and heat as compared with the control. Meanwhile, weight loss was reduced significantly by increasing the doses of irradiation.

Data in Table (3), show that weight loss percentage of Bartamoda date fruits. It is obvious that, as storage period extended, fruits weight loss also increased in all treatments of irradiation and methyl bromide treatments significantly reduced weight loss as compared with the control.

Concerning, irradiation doses it is clear that the highest dose caused the highest effect in decreasing fruit weight loss as compared with low doses. It is found that weight loss increased significantly by increasing storage period in all treatments. Meanwhile, irradiation treatments significantly reduced weight loss followed by methyl bromide and heat treatments as a compared with the control. However, weight loss increased significantly by decreasing the doses of irradiation.

These results are somewhat in agreement with the findings of (Emam *et al.,* 1994). They concluded that irradiation was more effective than methyl bromide since methyl bromide caused a significantly weight loss of semi- dray date fruits cv. El- Seidi. Besides, the findings of confirmed our results. (El-Salhy, Fatemah, 1998) on date palm Cultivars, (Vincent and Lingered 2002) and (Saharan & Mehta 2008) on date fruits.

4- Moisture content percentage:

Data in Table (4) clarifies that moisture content percentage of Sackoti date fruits decreased by increasing storage period in all treatments. However, methyl bromide treatment showed the lowest moisture content of dry date fruits followed by irradiation treatments as compared with the control.

Meanwhile, moisture content percentage was reduced significantly by increasing the doses of irradiation. In general, heat treatment significantly reduced in fruit moisture content as compared with either irradiation or methyl bromide. Comparing different doses of irradiation it is clear that there is an indirect relationship between irradiation dose and moisture content in the fruit.

A glance to table (4) data showed that moisture content of Bartamoda date fruit decreased by prolonging the storage period. Moreover, irradiation treatments were superior in decreasing moisture content than methyl bromide. Comparing different doses of irradiation it is quite evident that the highest doses (1.00KGy) were lower in this respect in comparison with the other doses which showed significant differences between them. These results agreed with those of (El- Badawy, 2001; Mathur, 2003; Romani 2004 and Mehaisen 2005 b).

5- Total sugars content percentage:

Table (5) show the effect of heat, methyl bromide and irradiation treatments on total sugars percentage of Sackoti date fruits. It is quite evident that in all treatments used total sugars percentage decreased with increasing storage period. Considering irradiation and methyl bromide treatments, it is clear that irradiation treatments succeeded significantly in increasing total sugars percentage as compared with methyl bromide and control. Comparing different doses of irradiation it is obvious that no significant difference was noticed between different doses of irradiation. Moreover, data indicated that, total sugars percentage of Sackoti date fruits decreased by the increase in storage period. Concerning irradiation, heat and methyl bromide treatments it is clear that these treatments had no statistical effect in this concern.

Besides, Table (5) show the effect of different treatments on total sugars percentage of Bartamoda date fruits.

It is found that, values of total sugars percentage decreased significantly by increasing storage period in all treatments. In general, irradiation treatments succeeded in reducing the loss in total sugars percentage followed by methyl bromide treatment in a decreasing order. Concerning irradiation, heat and methyl bromide treatments it is clear that all treatments decreased percentage of total sugars blow the control. Nevertheless, differences between these treatments was so small to be significant. Similar results obtained by (Hegazi *et al.,* 1989, Emam, *et al.,* 1994, Jaddou *et al.,* 2001, Mathur, 2003 and Saharan & Mehta 2008).

6- Reducing sugars percentage:

Data in table (6) show the effect of heat, methyl bromide and irradiation treatments on reducing sugars percentage of Sackoti date fruits. It is clear that in all treatments used reducing sugars percentage decreased with increasing storage period.

Considering irradiation and methyl bromide treatments it is found that methyl bromide treatment caused a decrease in reducing sugars percentage as compared with irradiation treatments and the control. Comparing different doses of irradiation, it is quite evident that both high (1.00KGy) and low dose (0.25 KGy) were superior in this respect.

Moreover, the effect of treatments on reducing sugars percentage o Bartamoda date fruits was shown in Table (6). It is found that, reducing sugars decreased significantly as storage period in all treatments.

Besides, irradiation treatments significantly maintained reducing sugars percentage at a higher level, as compared with the methyl bromide and control but with no statistical differences between different doses of irradiation.

These data were in line with those obtained by (Morell, 1991, Atwa, Marwa, 1999, De- Kock & Holz, 2001, Vincent 2002 and Saharan & Mehta2008).

7- Tannins Content Percentage:

Tannins percentage of Bartamoda as well as Sackoti fruits during two seasons, as affected by heat, methyl bromide and irradiation treatments are presented in table (7). Generally, tannins percentage were slightly affected by the different used treatments without any significantly between them.

The decreases in fruits tannins content during maturation and storage may be attributed to the fact that soluble leucocyadin tannins are converted during maturation into insoluble tannins, which take part in non enzymic oxidative browning thus insoluble leuco anthocyandin decrease during storage period (Maier and Metzer, 1965, A. O. A. C. 1990, Ying & Paulson 2000 and Raweewon 2008).

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Tables

Table (1): Associated fungi causing to infection and isolated inside and outside of date fruits.

Isolates fungal group	1st season (2010/2011)			
	cv. Sackoti		cv. Bartamoda	
	No. of date fruits	% isolation	No. of date fruits	% isolation
Penicillum expensium	96	32.0	105	35.0
Aspergillus niger	88	29.5	79	26.3
Rhizoups nigricans	60	20.0	63	21.2
Stemphyllium sp.	18	6.0	20	6.5
Mucor sp.	18	6.0	15	5.0
Geotrichum sp.	11	3.5	9	3.0
Trichothecium sp.	9	3.0	9	3.0
Total	**300**	**100**	**300**	**100**
2nd season (2011/2012)				
Penicillum expensium	99	33.0	114	38.0
Aspergillus niger	84	28.0	69	23.0
Rhizoups nigricans	57	19.0	61	20.5
Stemphyllium sp.	22	7.5	25	8.2
Mucor sp.	18	6.0	18	6.0
Geotrichum sp.	15	5.0	9	3.0
Trichothecium sp.	5	1.5	4	1.3
Total	**300**	**100**	**300**	**100**

Table (2): Effect of heat, methyl bromide and irradiation treatments on fruits infection percentage of dry datefruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti							cv. Bartamoda						
	1st season (2010/2011)													
	Storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	0.0	22.2	32.2	41.2	48.9	60.0	34.1A	0.0	20.0	36.7	43.3	60.0	70.0	38.3A
Heat at 40oC	0.0	17.8	21.1	39.0	50.1	60.0	31.3 B	0.0	18.1	26.3	41.0	53.5	65.0	34.0B
Methyl bromide	0.0	0.0	2.3	7.8	16.7	35.0	10.7 F	0.0	5.6	10.7	18.3	20.2	40.0	15.8F
0.25 KGy	0.0	13.3	27.7	30.0	36.7	38.3	24.3 C	0.0	17.0	27.0	31.3	43.5	60.7	30.0C
0.50 KGy	0.0	10.0	26.7	30.0	33.3	35.0	22.5D	0.0	19.2	25.1	30.3	38.2	50.0	27.1D
1.00 KGy	0.0	10.0	16.7	23.3	25.3	30.0	17.6E	0.0	15.6	22.6	25.5	30.0	32.5	21.0E
Mean	0.0F	12.4 E	21.1 D	28.6 C	35.2B	43.1 A	-	0.0F	15.9E	24.7D	31.6C	40.9B	53.0A	-
2nd season (2011/2012)														
Control	0.0	23.3	30.0	37.0	50.0	70.0	35.1A	0.0	27.0	40.0	50.0	56.7	70.0	40.6A
Heat at 40oC	0.0	20.0	26.7	40.0	50.0	60.0	32.8B	0.0	20.0	26.2	35.0	40.0	46.7	28.0B
Methyl bromide	0.0	0.0	0.0	0.0	30.0	33.3	10.6E	0.0	7.7	11.3	16.5	26.7	30.0	15.7D
0.25 KGy	0.0	13.3	16.7	26.7	30.0	36.0	20.5C	0.0	16.3	26.4	33.5	41.0	55.0	28.7B
0.50 KGy	0.0	13.0	15.6	26.8	29.3	35.0	2`0.0C	0.0	20.0	27.0	31.5	40.1	51.7	28.4 B
1.00 KGy	0.0	12.0	12.8	20.1	25.1	28.2	16.4D	0.0	16.0	20.8	27.6	30.0	32.0	21.1C
Mean	0.0F	13.6 E	17.0D	25.1C	35.7B	43.8A	-	0.0F	17.8E	25.3D	32.4C	39.1B	47.6A	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Table (3): Effect of heat, methyl bromide and irradiation treatments on weight loss percentage of dry date fruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti							cv. Bartamoda						
	1st season (2010/2011)													
	Storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	0.0	1.5	3.1	4.6	6.1	8.7	4.00A	0.0	3.1	4.7	7.8	10.3	12.8	6.45A
Heat at 40oC	0.0	0.4	0.8	2.0	3.5	6.7	2.30DE	0.0	1.2	2.4	2.9	4.4	5.9	2.80C
Methyl bromide	0.0	0.8	1.4	2.1	4.0	6.1	2.40 D	0.0	2.1	2.5	3.0	3.5	5.2	2.72CD
0.25 KGy	0.0	1.2	1.6	2.7	5.8	7.3	3.10 B	0.0	1.9	2.3	2.8	4.9	6.7	3.10 B
0.50 KGy	0.0	1.0	1.4	2.1	5.6	6.9	2.83 C	0.0	1.3	1.8	2.7	4.7	6.3	2.80 C
1.00 KGy	0.0	0.8	1.2	1.9	4.8	6.0	2.45 D	0.0	0.8	1.4	2.1	3.9	5.4	2.27 D
Mean	0.0 F	0.95 E	1.58D	2.57C	4.97B	6.95A	-	0.0 F	1.73 E	2.52D	3.55C	5.28B	7.05A	-
2nd season (2011/2012)														
Control	0.0	1.5	2.3	2.7	3.7	6.6	2.80A	0.0	3.6	4.5	6.8	9.5	11.2	5.93 A
Heat at 40oC	0.0	0.2	0.9	2.3	3.7	6.8	2.32CD	0.0	1.6	2.5	3.8	4.5	6.2	3.10 B
Methyl bromide	0.0	1.4	1.9	2.7	4.5	5.1	2.70 B	0.0	2.0	2.6	3.1	4.1	5.3	2.85BC
0.25 KGy	0.0	1.8	1.4	3.0	5.2	5.1	2.75 AB	0.0	2.1	2.7	3.0	4.5	6.3	3.10 B
0.50 KGy	0.0	1.5	1.8	2.7	4.5	6.0	2.75AB	0.0	1.5	2.0	2.7	4.2	5.6	2.67 C
1.00 KGy	0.0	1.1	1.5	2.3	3.6	5.8	2.38C	0.0	1.0	1.6	2.0	3.8	5.1	2.5 D
Mean	0.0 F	1.25 E	1.63D	2.62C	4.20B	6.00A	-	0.0 F	1.97 E	2.65D	3.67C	5.10B	6.62A	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Table (4): Effect of heat, methyl bromide and irradiation treatments on moisture content percentage of dry date fruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti							cv. Bartamoda						
	1st season (2010/2011)													
	Storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	14.8	14.8	14.7	14.7	14.5	14.3	14.63A	18.7	18.4	18.4	18.3	18.2	18.1	18.75A
Heat at 40oC	13.8	13.7	13.6	13.5	13.3	13.1	13.50B	18.3	18.2	18.0	17.7	17.6	17.3	18.02B
Methyl bromide	13.2	13.1	13.0	12.9	12.8	12.7	12.95C	18.2	18.1	17.8	17.8	17.7	17.3	17.82C
0.25 KGy	14.8	14.7	14.6	14.4	14.3	14.3	14.52AB	18.6	18.4	18.3	18.3	18.1	18.0	18.28AB
0.50 KGy	13.7	13.6	13.5	13.4	13.3	13.2	13.45B	18.4	18.3	18.1	17.8	17.7	17.4	17.95CD
1.00 KGy	13.5	13.5	13.4	13.3	13.2	13.1	13.33B	18.3	18.1	18.0	17.7	17.5	17.3	17.82C
Mean	13.97A	13.90A	13.80AB	13.70B	13.57BC	13.45C	-	18.75A	18.25B	18.10B	17.95BC	17.80C	17.57D	-
2nd season (2011/2012)														
Control	15.4	15.2	15.1	15.0	14.8	14.7	15.03A	19.8	19.7	19.6	19.4	19.4	19.2	19.52A
Heat at 40oC	14.9	14.8	14.6	14.6	14.5	14.3	14.78B	19.6	19.4	19.3	19.2	19.1	19.1	19.28B
Methyl bromide	13.9	13.7	13.6	13.5	13.4	13.3	13.57D	19.4	19.2	19.0	18.8	18.6	18.5	18.92C
0.25 KGy	14.2	14.1	14.0	13.8	13.7	13.5	13.88C	19.6	19.6	19.5	19.5	19.3	19.2	19.45AB
0.50 KGy	13.9	13.8	13.7	13.6	13.6	13.4	13.67CD	19.5	19.3	19.1	19.0	19.0	19.0	19.15BC
1.00 KGy	13.7	13.5	13.3	13.2	13.2	13.1	13.35E	19.3	19.2	19.0	18.8	18.6	18.4	18.88CD
Mean	14.33A	14.18AB	14.05B	13.95BC	13.87BC	13.72C	-	19.53A	19.40AB	19.25B	19.12BC	19.00BC	18.90C	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Table (5): Effect of heat, methyl bromide and irradiation treatments on total sugar content percentage of dry date fruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti													
	1st season (2010/2011)													
	storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	69.8	69.6	69.7	69.9	70.1	70.2	69.88A	75.5	75.5	75.7	76.2	76.5	76.7	76.02A
Heat at 40oC	69.8	69.5	69.6	69.8	70.0	70.0	69.78AB	75.5	75.8	75.9	76.0	76.0	76.5	75.95B
Methyl bromide	69.8	69.5	69.2	69.2	69.0	69.0	69.30B	75.5	75.7	75.7	75.9	76.0	76.0	75.80BC
0.25 KGy	69.8	69.6	69.3	69.0	68.7	67.0	68.90C	75.5	75.2	75.0	75.7	74.2	74.0	74.67C
0.50 KGy	69.8	69.3	69.0	68.5	68.2	68.0	68.80CD	75.5	75.0	74.8	74.4	74.0	73.6	74.55CD
1.00 KGy	69.8	69.2	68.7	68.2	67.8	67.3	68.52D	75.5	75.0	74.6	74.2	74.0	73.5	74.47D
Mean	69.80A	69.45AB	69.25AB	69.10B	68.97BC	68.58C	-	75.50A	75.37AB	75.28AB	75.23AB	75.12B	75.05C	-
2nd season (2011/2012)														
Control	70.5	70.6	70.9	71.0	71.2	71.4	70.93A	80.3	80.6	80.7	80.9	81.3	81.5	80.88A
Heat at 40oC	70.5	70.3	70.6	70.9	71.0	71.0	70.72AB	80.3	80.5	80.7	80.7	80.9	81.3	80.73AB
Methyl bromide	70.5	70.6	70.8	70.0	71.1	71.1	70.68B	80.3	80.0	80.2	80.5	80.5	81.2	80.45B
0.25 KGy	70.5	70.3	70.1	69.6	69.3	69.0	69.80BC	80.3	80.0	79.6	79.5	79.3	79.0	79.62BC
0.50 KGy	70.5	70.0	69.8	69.5	69.1	68.5	69.57BC	80.3	80.0	79.7	79.7	79.3	79.3	79.72BC
1.00 KGy	70.5	70.2	69.7	69.3	69.0	68.3	69.50C	80.3	79.8	79.5	79.2	79.0	79.6	79.07C
Mean	70.50A	70.33B	70.32B	70.05C	70.12CD	69.88D	-	80.30A	80.15AB	80.07B	80.08B	80.05B	80.32C	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Table (6): Effect of heat, methyl bromide and irradiation treatments on reducing sugar content percentage of dry date fruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti													
	1st season (2010/2011)													
	Storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	16.9	16.8	16.5	16.5	16.4	16.1	16.53A	35.6	35.6	35.6	35.3	35.0	34.5	35.27A
Heat at 40oC	16.9	16.8	16.5	16.3	16.4	16.1	16.50AB	35.7	35.6	35.3	35.0	35.0	34.6	35.20AB
Methyl bromide	16.8	16.5	16.5	16.5	16.4	16.0	16.45AB	35.8	35.6	35.5	35.3	34.7	34.2	35.18AB
0.25 KGy	16.6	16.6	16.5	16.5	16.5	16.0	16.45AB	35.9	35.6	35.4	34.9	33.9	33.0	34.80B
0.50 KGy	16.8	16.8	16.5	16.2	16.2	15.8	16.38B	35.8	35.5	35.4	33.8	33.5	33.2	34.53BC
1.00 KGy	16.6	16.5	16.4	16.0	15.8	15.5	16.13C	35.3	35.0	35.6	34.2	33.8	33.2	34.35C
Mean	16.60AB	16.67A	16.48AB	16.33AB	16.28B	15.92C	-	35.68A	35.48AB	35.30B	34.75BC	34.32C	33.80D	-
2nd season (2011/2012)														
Control	16.9	16.8	16.8	16.4	16.4	16.2	16.58A	35.7	35.6	35.2	34.4	34.4	33.1	34.73A
Heat at 40oC	16.9	16.9	16.7	16.4	16.2	16.1	16.53AB	35.4	35.2	35.1	34.4	34.5	33.6	34.72A
Methyl bromide	16.9	16.8	16.5	16.5	16.4	16.0	16.52AB	35.0	35.0	35.0	34.7	34.3	32.8	34.47AB
0.25 KGy	16.8	16.8	16.7	16.3	16.3	16.1	16.50AB	34.9	34.8	34.8	34.7	34.1	33.0	34.38B
0.50 KGy	16.9	16.8	16.5	16.3	16.4	16.1	16.50AB	35.3	25.1	34.2	34.4	33.9	32.6	34.25BC
1.00 KGy	16.2	16.8	16.6	16.3	16.3	16.1	16.38B	35.0	34.8	34.8	34.1	33.1	32.9	34.12C
Mean	16.90A	16.82AB	16.63B	16.37BC	16.33C	16.10D	-	35.22A	35.10AB	34.85B	34.45BC	34.05C	33.00D	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Table (7): Effect of heat, methyl bromide and irradiation treatments on tannins content percentage of dry date fruits (cvs. Sackoti and Bartamoda) during storage periods.

Treatment	cv. Sackoti													
	1st season (2010/2011)													
	Storage periods (months after treatment)													
	Start	2	4	6	8	10	Mean	Start	2	4	6	8	10	Mean
Control	0.27	0.25	0.23	0.20	0.19	0.19	0.22A	0.21	0.20	0.19	0.18	0.17	0.16	0.19A
Heat at 40oC	0.23	0.21	0.20	0.20	0.20	0.19	0.21AB	0.20	0.19	0.15	0.14	0.14	0.13	0.16B
Methyl bromide	0.21	0.20	0.20	0.20	0.19	0.18	0.20AB	0.20	0.15	0.14	0.13	0.14	0.14	0.15BC
0.25 KGy	0.22	0.20	0.19	0.18	0.20	0.20	0.20AB	0.19	0.18	0.13	0.14	0.13	0.11	0.15BC
0.50 KGy	0.20	0.18	0.21	0.20	0.19	0.18	0.19B	0.19	0.18	0.14	0.13	0.13	0.12	0.15BC
1.00 KGy	0.19	0.19	0.18	0.18	0.19	0.18	0.19B	0.19	0.17	0.15	0.12	0.10	0.12	0.14C
Mean	0.22A	0.21AB	0.20AB	0.19B	0.19B	0.19B	-	0.20A	0.18B	0.15CD	0.16C	0.14CD	0.13D	-
2nd season (2011/2012)														
Control	0.25	0.22	0.19	0.18	0.20	0.18	0.20A	0.18	0.17	0.16	0.15	0.16	0.16	0.16A
Heat at 40oC	0.19	0.20	0.20	0.19	0.19	0.20	0.20A	0.17	0.16	0.13	0.14	0.14	0.15	0.15AB
Methyl bromide	0.21	0.20	0.20	0.18	0.20	0.18	0.20A	0.16	0.15	0.13	0.13	0.14	0.15	0.14B
0.25 KGy	0.19	0.18	0.20	0.19	0.18	0.18	0.19B	0.15	0.14	0.14	0.14	0.13	0.14	0.14B
0.50 KGy	0.20	0.18	0.19	0.18	0.18	0.19	0.19B	0.15	0.14	0.15	0.13	0.13	0.13	0.14B
1.00 KGy	0.21	0.20	0.19	0.18	0.19	0.18	0.19B	0.15	0.13	0.12	0.12	0.10	0.11	0.12C
Mean	0.21A	0.20AB	0.20AB	0.19B	0.19B	0.19B	-	0.16A	0.15AB	0.14B	0.14B	0.13BC	0.14B	-

Means followed by the same letter, within each column are not significantly different from each other at 1% level.

Date Palm Improvement

Cryopreservation of date palm meristematic cells

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is the most important perennial plant in sub-Saharan and hot regions. Genetic erosion is among the serious problems which date palm is facing. This study aimed to produce and cryopreserve meristematic cell aggregates having the capacity to generate adventitious buds or somatic embryos. A biochemical investigation was carried out to explain the utility of the sucrose preculture and the cold hardening phases in a cryopreservation protocol. MS medium supplemented with 70 g/L sucrose was effective to get meristematic cells having the capacity to generate buds or somatic embryos from in vitro tissue culture. Compared to the standard vitrification protocol, the encapsulation vitrification and particularly the ultra-rapid droplet freezing techniques proved their high efficiency for the cryopreservation of the date palm meristematic cells. Thus, the highest survival rates using these techniques were 26.7, 53.3 and 66.7 % respectively. The multiplication rates, measured after a cultivation period of 3 months, of control and cryopreserved plant material were 3 and 2 successively. Sucrose preculture and cold hardening which both could induce activation of genes coding for resistance towards osmotic stress, as observed in total soluble proteins profiles and proline content measurements, increased considerably post thaw recovery rates after vitrification.. We showed that cryopreservation does not affect the morphogenetic capacities of this plant material. Indeed, multiple bud or embryogenic suspension cultures were established. Morphological studies showed the genetic stability of clonal material following cryopreservation.

Key words: date palm, genetic resources, cryobiology, tissue culture, caulogenesis, embryogenesis, SDS PAGE, proline

INTRODUCTION

Socio-economically, one of the most important perennial plant in sub-Saharan and hot regions is date palm (*Phoenix dactylifera* L.). This is why extensive efforts have been undertaken by the scientific community to overcome constraints hampering the extension of date palm plantations (El Hadrami and El Hadrami 2009). Biotechnological tools are effective to propagate, improve and preserve plant genetic resources (Pati et al. 2006; Parveez et al. 2000; Engelmann 2004; Panis 2008). In case of date palm, biotechnologies have already been fully employed for large scale propagation (Fki et al. 2003; Fki et al. 2010). Nevertheless, biotechnological approaches for date palm improvement and preservation still need more investigations. This study aims to produce and cryopreserve meristematic cells having the capacity to generate true-to-type in vitro date palms. A biochemical study was carried out to explain the benefits of the sucrose preculture and the cold hardening phases in a cryopreservation protocol.

MATERIALS AND METHODS

Meristematic cell aggregates were initiated from date palm in vitro chlorophyll-free leaves using MS medium supplemented with 70 g/L sucrose (Murashige and Skoog 1962). Embryogenic suspensions were established from calli (0.5 g) and maintained on a rotary shaker at 100 rpm. For shoot multiplication, RITA bioreactors (Alvard et al. 1993) for the temporary immersion of cultures in liquid medium were used.

The RITA vessel is made of two compartments: the explants are cultivated in the upper compartment and the lower one holds the liquid medium. Six bud clusters per bioreactor were cultivated using 200 ml of MS medium supplemented with 70 g/L sucrose. The immersion cycle was 15 min every 24 h and the culture medium was renewed once every 4 months. Stock cultures were incubated in a growth chamber at 28 °C under a 16h photoperiod (photon flux: 30 µEm-2 s-1).

Prior to cryopreservation, explants (< 3 mm) bearing meristematic cell aggregates were cultured on MS medium enriched with 180 g l-1 sucrose or incubated at 4 °C for 2, 5 and 10 days. For cryopreservation, the standard vitrification, the encapsulation vitrification and the droplet vitrification protocols have been applied (Panis et al. 2005). Three different cryopreservation protocols were assessed in the present study, namely: standard (tube) vitrification, droplet-vitrification and encapsulation-vitrification. Three replicates of ten samples were used for each experiment.

In the first two protocols (standard vitrification and droplet-vitrification), explants were transferred into 15 cm3 loading solution (LS) containing 2 M glycerol and 0.4 M sucrose in MS medium for 20 min (Panis et al. 2005).

In the third protocol explants were placed into previously autoclaved 3 % (w/v) sodium alginate dissolved in MS medium, with 7 % (w/v) sucrose and no CaCl2; then they were sucked up with a micropipette and gently dropped into 75 mM CaCl2, 2 H2O in MS medium supplemented with 7 % sucrose (Lakshmana and Singh 1990) and kept for 15 min. Encapsulated plant tissues were then transferred into the loading solution for 20 min.

The loading solution was then replaced by ice-cooled PVS2 solution (Sakai et al. 1990). This solution consisted of 30% (3.26 M) glycerol, 15% (2.42 M) ethylene glycol (EG) and 15% (1.9 M) DMSO in MS medium containing 0.4 M sucrose. The pH was adjusted to 5.8 and the solution was filter sterilized. Both naked and encapsulated explants were treated with PVS2 solution for 15, 30, 60 or 120 min at 0 °C.

Explants were transferred into 2 ml cryotubes containing 0.5 ml PVS2 and then plunged into liquid nitrogen (Standard vitrification protocol). Alternatively, explants were transferred to a droplet of PVS2 on a strip of aluminium foil and then plunged into liquid nitrogen (droplet-vitrification protocol). For permanent cryostorage, frozen foil strips were quickly transferred to 2 ml cryotubes filled with liquid nitrogen then closed.

For encapsulated explants, alginate beads were transferred into 2 ml cryotubes filled with 0.5 ml PVS2 solution then plunged in liquid nitrogen (encapsulation-vitrification protocol).

After one hour of LN storage, strips of aluminium foil were transferred to recovery solution (RS) containing 1.2 M sucrose dissolved in MS medium for 15 min at room temperature (25 °C). Cryotubes containing the meristems or alginate beads were thawed in a water bath at 40 °C for 2 min then treated by RS at room temperature for 15 min. Explants were then placed onto two sterile filter papers on top of MS medium containing 180 g/L sucrose and then incubated in the dark. After 2 days, tissues were transferred onto MS medium containing 50 g/L sucrose and 0.1 mg/L 2,4-D. Survival rates were estimated using growth measurement at 4-6 weeks after thawing.

For histological examinations, explants were fixed in Svaloff Navashine solution (chromic acid 0.5 %, glacial acetic acid 5%, formaldehyde 15% and ethanol 5 %), then gradually dehydrated using ethanol solutions (50 to 100%) and finally embedded in paraffin. Serial sections (10 µm) were cut with a rotary microtome and stained with acetohematoxylin (Sass 1958).

For protein extraction, samples (0.5 g FW) were ground in liquid nitrogen then homogenized in 1 cm3 of maleate/Tris buffer 50 mM (pH 8.3) containing 2 % SDS, 0.5 mM EDTA, 2 mM PMSF, 1 mM DTT and 2 mM β-mercaptoethanol. Homogenates were centrifuged at 13,000 g for 15 min at 4 °C. Total soluble protein content of the supernatant was estimated according to Bradford (1976). For SDS-PAGE protein electrophoresis, samples (10 µg per lane) were loaded onto 12 % SDS gels and stained with Coomassie Brilliant Blue-R250 (Stone and Gifford 1997).

Proline content was estimated according to Bates et al. (1973) on 1 gFW of leaf tissue using 6 ml 3% sulfosalicylic acid. Two cm3 of the extract were placed for 1 h in boiling water with 2 cm3 ninhydrin and 2 cm3 glacial acetic acid and then cold toluene (4 ml) was added. Extracts were then filtered through a Whatman paper filter. Proline content was estimated spectrophotometrically at 520 nm and calculated as µmol/g against standard L-proline (Sigma-Germany P-0380).

Statistical analyses of data were performed using one-way ANOVA and Duncan's test. *P* values < 0.05 were considered as statistically significant. Statistical analysis was computed using SPSS 13 software. Experiments were replicated three times. Data expressed in percentage were transformed by arcsin transformation and then analyzed. Arcsin transformation (y' = arcsin y ½, y = original percentage/100) was undertaken in order to stabilize the variance of data.

RESULTS

Murashige and Skoog medium supplemented with 70 g/L sucrose was effective to generate date palm meristematic cells from in vitro tissue culture. Hypertrophied

chlorophyll-free leaves showed a high morphogentic capacity as they produced number of meristematic cell aggregates after only 3 months (Fig. 1).

Compared to the standard vitrification protocol, the encapsulation vitrification and particularly the ultra-rapid droplet freezing techniques proved their high efficiency for the cryopreservation of the date palm meristematic cells. Thus, the highest regeneration rates using these techniques were 26.7, 53.3 and 66.7 %, respectively (Table 1). Sucrose preculture and cold hardening both improved considerably post thaw recovery rates after vitrification. Both treatments were found to increase proline contents (Table 2) and to change the expression of 15 and 18 kDa proteins (Fig. 2). Besides, a newly expressed 21 kDa protein was detected only after cold hardening (Fig. 2). We also showed that cryopreservation does not affect the morphogenetic capacities of this plant material. Indeed, cryopreserved meristematic cells could produce proembryos or adventitious buds. Furthermore, multiple bud cultures and embryogenic suspension cultures were established employing temporary immersion system (TIS) and agitated liquid media, respectively. With respect to the effect of the cryogenic treatments on the genetic integrity, no morphological differences were observed between plants regenerated from non-cryopreserved controls and cryopreserved meristematic cells. All the plants showed a similar growth rate in the greenhouse (0.5 ± 0.2 cm in length per month), leaf colour and morphology. These observations are encouraging as regards genetic stability of cryopreserved material.

DISCUSSION

In this paper, we showed that cryopreservation of meristematic cells is a promising tool to establish date palm cryobanks. *In vitro* generated chlorophyll-free leaves were found to be a choice material to get meristematic cells. Enhancing sucrose concentration in the medium was sufficient for cell dedifferentiation. Our previous studies showed that PGRs such as 2,4-D were essential for cells dedifferentiation within primary explants tissue and that the culture period required to observe neoformations was much longer, especially when low concentrations of 2,4-D were used (Drira, 1983; Fki et al. 2011*a*). Removing PGRs from culture media can minimise the risk of both somaclonal variation and loss of morphogenetic capacity (Bairu et al. 2011). Indeed, LoSchiavo et al. (1989) showed that auxins impact global DNA methylation rates which might disturb gene expression and phenotype. Fki et al. (2011b) confirmed that high level of PGRs was the cause of somaclonal variation in date palm.

We proved the benefits of the sucrose preculture and the cold hardening on post-thaw regeneration. Both treatments seem to be effective to activate genes coding for resistance towards severe osmotic stress and ultra-low temperature. Basic knowledge about cryoprotection is improving fast: indeed, the determination of physical and biochemical changes associated with tolerance to cryopreservation is a very interesting approach to optimize cryopreservation protocols (Kaviani 2011). We carried out such a biochemical study in order to assess the effect of sucrose preculture and cold hardening on the total soluble protein profiles and proline content of explants. Helliot et al. (2003) monitored ultra-structural changes occurring during the cryopreservation of banana apical meristems. Moreover, differential scanning calorimetry (DSC) was used to discover the principal thermal events connected with plant cryopreservation procedures (Nadarajan et al. 2008, Sisunandar et al. 2010). The impact of sucrose preculture on protein metabolism in banana meristems was studied by Carpentier et al. (2010) through 2-D gel electrophoresis. These authors demonstrated that preculture was able to modulate the expression of genes which are essential for the acquisition of freezing tolerance. On the other hand, Zhu et al. (2006) demonstrated that sucrose pretreatment induced changes in sugar, sterol and fatty acid composition in banana meristems.

Many reports showed the efficiency of the vitrification technique and its two derived protocols, encapsulation-vitrification and droplet-vitrification (see Sakai and Engelmann 2007, for a review). In this study, we concluded that droplet vitrification remains the best way for date palm germplasm cryobanking.

Acknowledgment

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Tables

Table 1: Regeneration (%) of meristematic cell aggregates after 0 to 120 min exposure to PVS2 solution at 0 °C, followed (+LN) or not (-LN) by cryopreservation. Ten samples were used in each of the three replicates. +LN: cryopreserved meristems; -LN: non cryopreserved; PC: sucrose preculture (2 days, sucrose: 180 g/L); CH: cold hardening (2 days, at 4 °C) v: standard vitrification; ev: encapsulation-vitrification; dv: droplet-vitrification. Data within a column with the same letters are not significantly different according to Duncan's test after arcsin transformation ($P < 0.05$).

Regeneration (%)												
PVS2 exposure at 0 °C (min)	-LN -PC -CH	-LN +PC	-LN +CH	+LN v -PC -CH	+LN v +PC	+LN v +CH	+LN ev -PC -CH	+LN ev +PC	+LN ev +CH	+LN dv -PC -CH	+LN dv +PC	+LN dv +CH
0	96.7a	93.3a	96.7a	0a	0a	0 a	0a	0a	0a	0a	0a	0a
15	93.3a	96.7a	93.3a	6.7b	16.7c	6.7b	0a	0a	0a	13.3c	46.7d	36.7d
30	93.3a	96.7a	93.3a	13.3c	26.7d	23.3d	13.3b	23.3b	16.7b	36.7e	66.7e	53.3e
60	93.3a	93.3a	93.3a	6.7b	6.7b	6.7b	26.7cd	53.3d	46.7cd	16.7d	26.7c	23.3c
120	96.7a	93.3a	93.3a	0a	0a	0a	23.3c	33.3c	43.3c	6.7b	13.3b	6.7b

Table 2: Effect of sucrose (180 g/L) and cold (4 °C) treatments on proline content in date palm leaf tissue bearing meristematic cells. Experiments were replicated three times. Data followed by the same letter within the same column are not significantly different according to Duncan's test ($P < 0.05$)

Duration of the treatment (days)	Proline content (µg proline per g FW)	
	Sucrose (180 g/L) Treatment	Cold (4 °C) Treatment
0	99,3 a	99 a
2	370 b	376 b
5	373,3 b	365 b
10	368,3 b	378,3 b

Figures

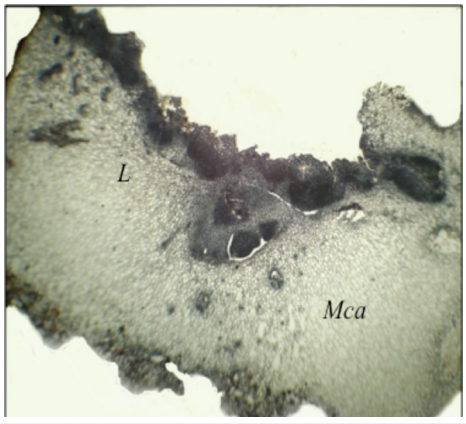

Fig. 1. Cross section showing meristematic cell aggregates within *in vitro* date palm chlorophyll-free leaf. *Mca* meristematic cell agregate, *L* hypertrophied chlorophyll-free leaf. *Scale bar* 1 mm

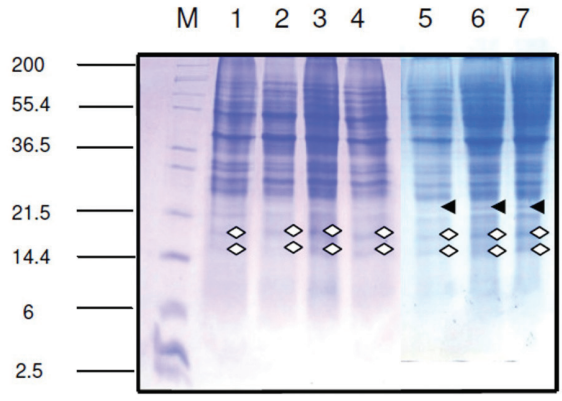

Fig. 2. Effects of sucrose preculture and cold hardening on the total soluble proteins profiles of the highly proliferating meristems. M: marker; Lane 1: control; Lane 2: 2days 180 g/L sucrose; Lane 3: 5 days 180 g/L sucrose; Lane 4: 10 days 180 g/L sucrose; Lane 5: 2 days 4°C; Lane 6: 5 days 4°C; Lane 7: 10 days 4 °C

Molecular characterization of date palm (Phoenix Dactylifera L.) using Inter Simple Sequence Repeat (ISSR) markers

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ABSTRACT

To study the genetic diversity among date palm cultivars grown in Qatar, fifteen Date palm samples were collected from Qatar University Experimental Farm. DNAs were extracted from fresh leaves by using commercial DNeasy Plant System Kit (Qiagen, Inc., Valencia, CA). Total of 18 (Inter Simple Sequence Repeat) ISSR single primers were used to amplify DNA fragments using genomic DNA of the 15 samples. First screening was done to test the ability of these primers to amplify clear bands using Date palm genomic DNA. All 18 ISSR primers successfully produced clear bands in the first screening. Then, each primer was used separately to genotype the whole set of 15 Date palm samples. Total of 4794 bands were generated using 18 ISSR primers for the 15 Date palm samples. On average, each primer generated 400 bands. The Number of amplified bands varied from cultivar to cultivar. The highest number of bands was obtained using Primers 2, 5 and 12 for the 15 (470 bands), while the lowest number of bands were obtained by Primers 1, 7 and 8 where they produced only 329 bands. Markers were scored for the presence and absence of the corresponding band among the different cultivars. Data were subjected to cluster analysis. A similarity matrix was constructed and the similarity values were used for cluster analysis.

Key words: Date palm, ISSR (Inter Simple Sequence Repeat), Molecular diversity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), 2n=36, is a dioecious long-lived monocotyledonous tree, it belongs to the family Arecaceae. Date palm is an excellent crop in arid and semiarid regions of the world with high tolerance to environmental stresses (Adawy *et al.,* 2004). The annual world production of dates has reached 6-8 million mt (metric tons), representing a market exchange value of over 1 billion USD. Date palm is one of the most important horticultural crops in Qatar and is also used as an ornamental or shade plant in parks, gardens and alongside roads. Date Palm plantations represent 71% from the total area planted with fruit trees. Total area cultivated approximately 1366 ha (Containing 335765 trees bearing fruits and 146955 non-productive trees). Most cultivation are 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).

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 (Baaziz 2000; Sedra *et al.,* 1998); Suadi Arabia (Al-Khalifah and Askari, 2003); Tunisia (Trifi *et al.,* 2000; Zehdi *et al.,* 2004a,b); Sudan (Elshibli and Korpelainen, 2007).

Development of accurate fingerprint characterizing the common cultivars of date palm would be of great value in improvement of this important crop. Some molecular markers as ISSR (Adawy *et al.,* 2004), RAPD (Adawy *et al.,* 2004; Ben Abdallah *et al.,* 2000; El-Rayes., 2009; Trifi et al, 2000), AFLP (Adawy *et al.,* 2004; El-Khisin *et al.,* 2003), and SSR (Ahmed and Al-Qaradawi, 2009) were used to describe genotypes of date palm. In Saudi Arabia RAPD fingerprint was used to investigate the genetic diversity of 5 varieties. Of 20 primers, only 12 primers were replicated and 64 bands were produced. The profile was used to distinguish variety (Al-Moshile *et al.,* 2004). Based on two by two comparisons of the products, the genetic similarity was calculated in the region 70 to 85%. Cluster analysis and dendrogram were done by UPGMA and the cultivars were divided into two groups (Al-Moshile *et al.,* 2004). Moghaieb et al. (2010) investigated the genetic diversity and sex determination in 6 genotypes of male and female date palms by RAPD and ISSR markers. Polymorphism amount of the markers was 60.2 and 73% for RAPD and ISSR markers, respectively. The cluster analysis showed that unknown cultivars had close relation with Frehi,

The objective of this study is to study the genetic diversity among and within date palm cultivars grown in two different locations in Qatar using 18 (Inter Simple Sequence Repeat) ISSR markers.

MATERIALS AND METHODS

Plant materials

Forty seven date palm samples representing 15 cultivars from two gene bank collections (Rodat Alfaras Germplasm field and Germplasm field of Qatar University Experimental farm) were collected. 29 samples representing 11 varieties were collected from Rodat Alfaras Farm. Eighteen samples including six varieties were collected from Qatar University Experimental Farm (Table 1).

DNA extraction

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 100V for 30 min. The gels were stained in Ethidium bromide and visualized under UV light.

PCR amplification and ISSR assay

A total of 18 primers were tested to amplify the isolated DNA. These primers listed in Table 2, and their composition has been arbitrarily established. For PCR amplifications, a 25 µl reaction mixture was used and it contained between 20 and 30 ng of total cellular DNA (1 µl), 60 pg of primer (1 µl), 2.5 µl of 10X Taq DNA polymerase reaction buffer, 1.5 unit of Taq DNA polymerase (Quantum-Appligène, France) and 200 mM of each dNTP. . 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 1.5 % agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed using Gel documentation system.

Data analysis

Bands were precisely measured by 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).

RESULTS AND DISCUSSION

Total of 18 (Inter Simple Sequence Repeat) ISSR single primers were used to amplify DNA fragments using genomic DNA of the 15 samples. First screening was done to test the ability of these primers to amplify clear bands using Date palm genomic DNA. All the18 ISSR primers successfully produced clear bands in the first screening. Then, each primer was used separately to genotype the whole set of 15 Date palm samples (An example is shown in Figure 1). Total of 4794 bands were generated using the 18 ISSR primers for the 15 Date palm date palm cultivars. On average, each primer generated 400 bands. The number of amplified bands varied from cultivar to cultivar. The highest number of bands was obtained using Primers 2, 5 and 12 for the 15 cultivars, while the lowest number of bands was obtained by Primers 1, 7 and 8 where they produced only 329 bands. However, variation within each individual cultivar as number of polymorphic fragments was considerably smaller than the inter-specific variation among the studied cultivars.

Interestingly, thirty distinct unique bands were obtained to represent nine date palm cultivars. Four out of seven different sizes bands obtained from primer 3 were appeared in Hatamy, Barhee, Khadraway, and Thuri. Each band represents one cultivar. In 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 band was shown in Succary, Abu Main, Barhee, Naboot Saif and Khanezy.

Band pattern data was converted into a binary data and was analyzed using 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 KHUSH ZABAD and KHANEZY cultivars (0.750) which seem to be the nearest two varieties and can be closely regrouped. The following nearest two cultivars are found between ABU-MAIN and SHESHY. 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 fifteen studied Qatari date palm genotypes. The Dendrogram shown in Figure 2, illustrates the divergence between the studied Qatari date palm cultivars and suggests their tree branching.

Hatamy cultivar was in a separate far group compared to the rest of cultivars. ABU-MAIN and SHESHY; HELALY and KHALAS may constitute paired clusters (Fig. 2).

DNA markers are 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 ISSRs system compare to other systems 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 markers have been used to assess 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 SSR markers to detect the genetic diversity in date palm. We may 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 closed and could be considered as they came from one origin. Some cultivars were grouped in different similar pairs.

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Figures:

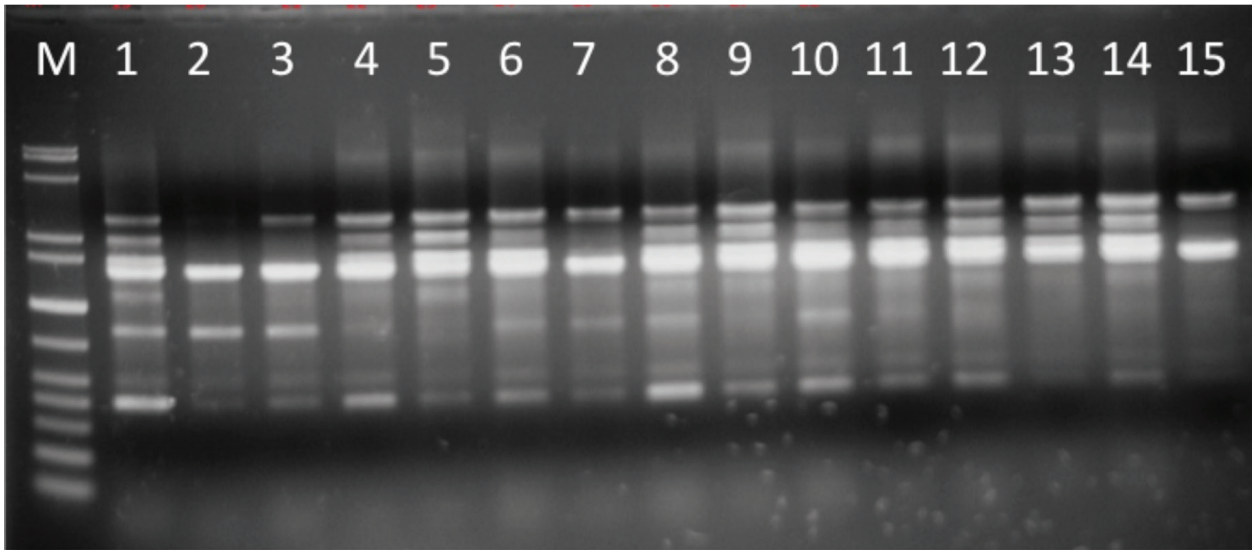

Figure 1. Example of ISSR polymorphism banding patterns in a subset of 15 Qatari date palm genotypes using primers # 10. M: 50 bp. Standard ladder marker; Lanes (1-15): Qatari Date palm genotypes described in Table 1.

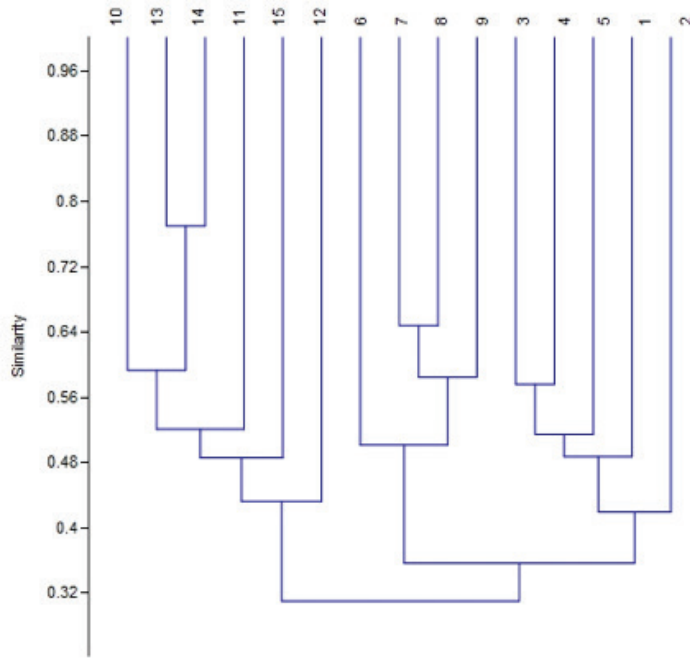

Figure 2. Dendrogram of 15 Qatari date-palm cultivars based on Jaccard genetic similarity coefficient using ISSR data. 1. ZAHIDI, 2. HATAMY, 3. HELALY, 4. KHALAS, 5. SUCCARY, 6. ANBARA, 7. ABU-MAIN, 8. SHESHY, 9. BARHEE, 10. SULTANA, 11. NABOOT-SAIF, 12. KHADRAWY, 13. KHUSH ZABAD, 14. KHANEZY and 15. THURI.

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	55^0 C
2	844A	(CT) 8AC	54^0 C
3	17898A	(CA)6AC	42^0 C
4	17898B	(CA)6GT	42^0 C
5	17899A	(CA)6AG	42^0 C
6	17899B	(CA)6GG	44^0 C
7	HB8	(GA)6GG	44^0 C
8	HB9	(GT)6GG	44^0 C
9	HB11	(GT)6CC	44^0 C
10	HB12	(CAC)3GC	42^0 C
11	844B	(CT)8GC	54^0 C
12	HB10	(GA)6CC	44^0 C
13	HB13	(GAG)3GC	38^0 C
14	HB14	(CTC)3GC	38^0 C
15	HB15	(GTG)3GC	38^0 C
16	TA-1	(AG)10C	62^0 C
17	TA-2	(CT)10G	62^0 C
18	TA-3	(AGG)6	62^0 C

DNA fingerprinting of some Iraqi date palm (*Phoenix dactylifera* L.) cultivars

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ABSTRACT

Date palm is the most important fruit tree in Iraq. DNA markers are a powerful tool to provide information on the relatedness of various clones or varieties that are difficult to distinguish morphologically. Five Iraqi date palm cultivars (Maktoum, Khidrawi Mandli, Osta Emran, Teberzal and Breem) were assessed using 23 RAPD markers. The results revealed that % of polymorphism ranged from 0 (primer OPC4,OPC14 and OPR7) to 85.7% (primer OPA5). One unique single band was shown in Breem variety obtained from primer OPE13, OPH7 and OPS12 in 800, 320 and 750 bp respectively, in Khidrawi Mandli variety from primer OPA8 and OPA11 at 320 and 500 respectively, in Teberzal variety from primer OPA17and OPH9 at 250 and 1100 bp respectively and in Osta Emran variety from primer OPA20 at 250 and 550bp, from primer OPC15 at 320 bp from primer OPF2 at 700 bp and from primer OPF8 at 400 and 580bp. Our results provide evidence of ability of RAPD markers to detect a genetic diversity among the tested date cultivars and this methodology can be extended to other cultivars.

INTRODUCTION

A variety of morphological characters of date fruits like shape, size, weight, color, texture, *etc*., have earlier been employed for the identification of date fruits, however discrimination among closely related cultivars by using fruit morphology traits are often unreliable and extremely difficult because of the influence of environmental conditions (Elhoumaizi *et al.,* 2002) and can be observed only in mature trees. Although biochemical studies including protein markers, isozyme analyses and activity analyses have been used to characterize date palms (Baaziz and Saaidi, 1988; Baaziz, 1988; Bendiab *et al.,* 1998), protein markers have been largely replaced by DNA-based approaches, mainly due to the fact that protein markers are limited in number and are dependence of their expression on environmental conditions (influenced by different environments as well as the developmental stage of the plant), and often-limited amount of detectable polymorphism (Winter and Kahl, 1995; Kunert *et al.,* 2001).

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. Several marker systems have been used widely and efficiently to analyze the genetic diversity within and among date palm cultivars like randomly amplified polymorphic DNA, RAPD (Al-Khalifah *et al.,* 2012), Amplified fragment length polymorphic AFLP (Jubrail *et al.,* 2005), inter simple sequence repeats –ISSR (Ahmed and Al-Qaradawi, 2010), microsatellites –SSR (Elshibli and Korpelainen, 2008) and restriction fragment length polymorphisms (RFLP) markers. Each marker type has specific advantages and disadvantages and their applications vary depending on the nature and objective of the investigation and the properties of the species. For genetic diversity studies, the RAPD technique shows some important advantages in date palm such as easier and faster way and simplest test technically (Abdulla and Gamal, 2010). Hence, several studies implied RAPD for the molecular characterization of date palm of Egypt (Soliman *et al.,* 2003 and Eissa *et al* .,2009, Sakr *et al.,* 2012), Iraq (Ali *et al.,* 2007), Syria (Haider

et al., 2012), Tunisia (Trifi *et al.,* 2000), Moroco (Sedra *et al.,* 1998), Saudi Arabia (Abdulla and Gamal 2010).

There are up to 5,000 date palm cultivars all around the world (Jaradat and Zaid 2004). Based on botanical descriptions, there are more than 600 cultivars in Iraq and the most important commercial varieties which representing 85% of the number of palm trees are Zahdi, Sayer, Helawi and Khidrawi and the rest (15%) including the most important and rare are Barhi, Breem, khistawi, Maktoum, Ashrasi, Al Cabcab, Deri, Teberzal, Hasawi, Ashger and Um Al Dehin.

Despite the large number of Iraqi varieties, little research has been undertaken on Iraqi date palm varieties depends on molecular markers. Among the marker system tested, employ PCR-RAPD markers for the early detection of genetic variations in *in vitro* culture-derived plants for Maktoum and Barhi varieties (Ali *et al.,* 2007; Bader *et al.,* 2007) and AFLP and SSR markers (Jubrail *et al.,* 2005 and Hamwieh *et al.,* 2010) for testing the genetic relatedness, this shows the lack of research on the DNA fingerprint of the Iraqi varieties. The objective of this study, therefore, was to assess PCR-RAPD markers which could be used in cultivar identification

MATERIAL AND METHODS

Date palm samples were collected from the Date Palm Experimental Station at Al Zufaranya, Ministry of Agriculture, Iraq during 2013 season. The samples are five female cultivars (Maktoum, Khidrawi Mandli, Osta Emran, Teberzal and Breem). The experiment was conducted in Agricultural Research Directorate, Ministry of Science & Technology, Baghdad- Iraq. Leaf tissue was ground to a fine powder, then 600 µl of CTAB extraction buffer (2% CTAB, 0.7M NaCl, 0.1M Tris-HCl pH 8, 20 mM EDTA and 1% β-mercaptoethanol) were added, mixed well and incubated at 60°C in a water bath. After 30 min of incubation with gentle swirling, the resulting cell lyses were extracted with 400 µl of chloroform / isoamyl alcohol (24:1, v/v). The cell lysate was then centrifuged at 13000 rpm for 15 min. The aqueous phase was transferred into another tube and precipitation occurred with the addition of 600 µl of isopropanol. The precipitate was then collected by centrifugation at 13000 rpm for 15 min. Pellets were washed with 70% ethanol, dried and dissolved overnight at 4°C in 50 µl of TE buffer (10mM Tris – HCl pH 8.0, 1mM EDTA). DNA concentration was read with Nano-Drop spectrophotometer (Bio-Rad, USA). A total of 23 random decamer primers (OPERON Model) manufactured by Bioneer-Korea were used. PCR was performed using an AccuPower©PCR Premix (Bioneer, Korea), containing 250 µM of each deoxyribonucleoside triphosphate, 30 mM of KCl, 10 mM of Tris- HCl (pH 9.0), 1.5 mM of MgCl2, and 1 Unit of Top DNA polymerase. 100 ng of genomic DNA and 100 ng of RAPD primer were then add to a PCR Premix tube. Amplification was performed in Thermocycler (FlexCycler, Germany) using program for: I cycle at 94°C for 4 min, 40 cycles as follows: 94°C for 45 sec, 36°C for 1 min, 72°C for 2 min, the last cycle at 72 °C for 10 min. Amplification products were loaded on 1% agarose gels and stained with ethidium bromide (0.5 mg/ml). The DNA banding patterns were visualized on an UV transilluminator and documented by using Gel Documentation System, E-Graph (AE-9000, Japan). Fragment length was estimated by comparison with standard size markers (100 bp DNA Ladder Size range (bp): 100 - 2000, Bioneer-Korea). Fragments (bands) were recorded numerically as (1) when present or (0) when absent. Fragments with the same mobility were considered as identical, irrespective of fragment intensity. Bands pattern data were analyzed using the SPSS 12 program to calculate similarity coefficient values according to Jaccard (1908).

REDULT AND DISCUTION

DNA of five Iraqi date palm cultivars was isolated from the leaf and amplified by PCR using 23 random oligonucleotide primers. Amplification products were separated by agarose gel electrophoresis to reveal band polymorphism. The result showed (Table1) that all primers produced clear reproducible bands and yielded 793 bands. The number of bands from each primer varied from 17 to 69, the primer OPA8 produced 69 fragments whereas, primer OPE13 produced 17 bands, primers showed polymorphic lines that ranged from 0 (primer OPC4,OPC14 and OPR7) to 85.7% (primer OPA5). RAPD variation has also been reported in many studies. For example, Haider *et al*. (2012) reported that % of polymorphism for syrian date palm varieties ranged from 0 to 92% while Saker *et al*. (2012) found that % of polymorphism of Egyptian date palm varieties ranged from 4.25 to 9.52.

In profiles generated, the sizes of the fragments ranged from 100 to 2000 bp. On the other hand, one unique single band was shown in Breem variety obtained from primer OPE13, OPH7 and OPS12 (Fig.1) in 800, 320 and 750 bp respectively, in Khidrawi Mandli variety obtained from primer OPA8 and OPA11 (Fig. 2) at 320 and 500 respectively, in Teberzal variety obtained from primer OPA17(Fig.3) and OPH9 at 250 and 1100 bp respectively and in Osta Emran variety from primer OPA20 at 250 and 550bp, from primer OPC15 at 320 bp (Fig.3), from primer OPF2 at 700 bp and from primer OPF8 at 400 and 580bp. Unique single bands thus can be used for the DNA fingerprinting, this confirms findings of Al-Khalifah and Askari (2003).

Band pattern data analyzed using the SPSS 12 program to calculate similarity coefficient values according to Jaccard (1908). A similarity matrix between Iraqi date palm cultivars showed an average similarity coefficient range from 0.631 to 0.785 (Table2). The highest similarity coefficient value was observed between Breem and Kadrawi Mandily which seem to be the nearest two varieties and can be closely

regrouped. The similarity matrices were used in the cluster analyses which were employed to generate dendrograms. The dendrogram shown in Figure 4, illustrates the divergence between the studied Iraqi date palm cultivars and suggests their tree branching which provide evidence of divergence among all tested genotypes since they were grouped in clusters. This confirms findings of many studies (Al-Khalifah *et al.,* 2012; Haider *et al.,* 2012; Sakr *et al.,* 2012, Sedra *et al.,* 1998), which found that RAPD markers are a powerful tool to provide information on the relatedness of various date palm varieties that are difficult to distinguish morphologically, therefore molecular marker might be the easier criteria to distinguished date palm cultivars

CONCLUSION

RAPD markers have been used to assess the molecular characterization and relationships of Iraqi date palm cultivars. Our results provide evidence of ability of RAPD markers to detect a genetic diversity among the tested date cultivars. The methodology followed in this study also can be extended to other cultivars

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Tables

Table1: RAPD-PCR amplification products of five date palm cultivars using 23random primers

Primer	Total number of band	Number of polymorphic band	Polymorphism (%)
OPA5	35	30	85.7
OPA8	69	22	31.9
OPA11	31	11	35.5
OPA15	27	2	7.4
OPA17	67	17	25.4
OPA20	47	27	57.4
OPB5	28	8	28.6
OPB10	21	9	42.9
OPC4	45	0	0.0
OPC15	55	0	0.0
OPD2	33	8	24.2
OPE8	40	15	37.5
OPE13	19	4	21.1
OPF2	20	5	25.0
OPF8	24	9	37.5
OPF12	37	2	5.4
OPH7	17	12	70.6
OPH9	37	27	73.0
OPH15	35	15	42.9
OPO16	37	12	32.4
OPR7	35	0	0.0

Primer	Total number of band	Number of polymorphic band	Polymorphism (%)
OPS12	35	15	42.9
OPZ11	23	3	13.0

Table2: Similarity matrix of 5 date palm varieties obtained from RAPD markers

Varieties	Osta Emran	Teberzal	Maktom	Kadrawi Mandily
Breem	0.674	0.698	0.784	0.785
Kadrawi Mandily	0.631	0.701	0.797	
Maktom	0.696	0.729		
Teberzal	0.702			

Figures

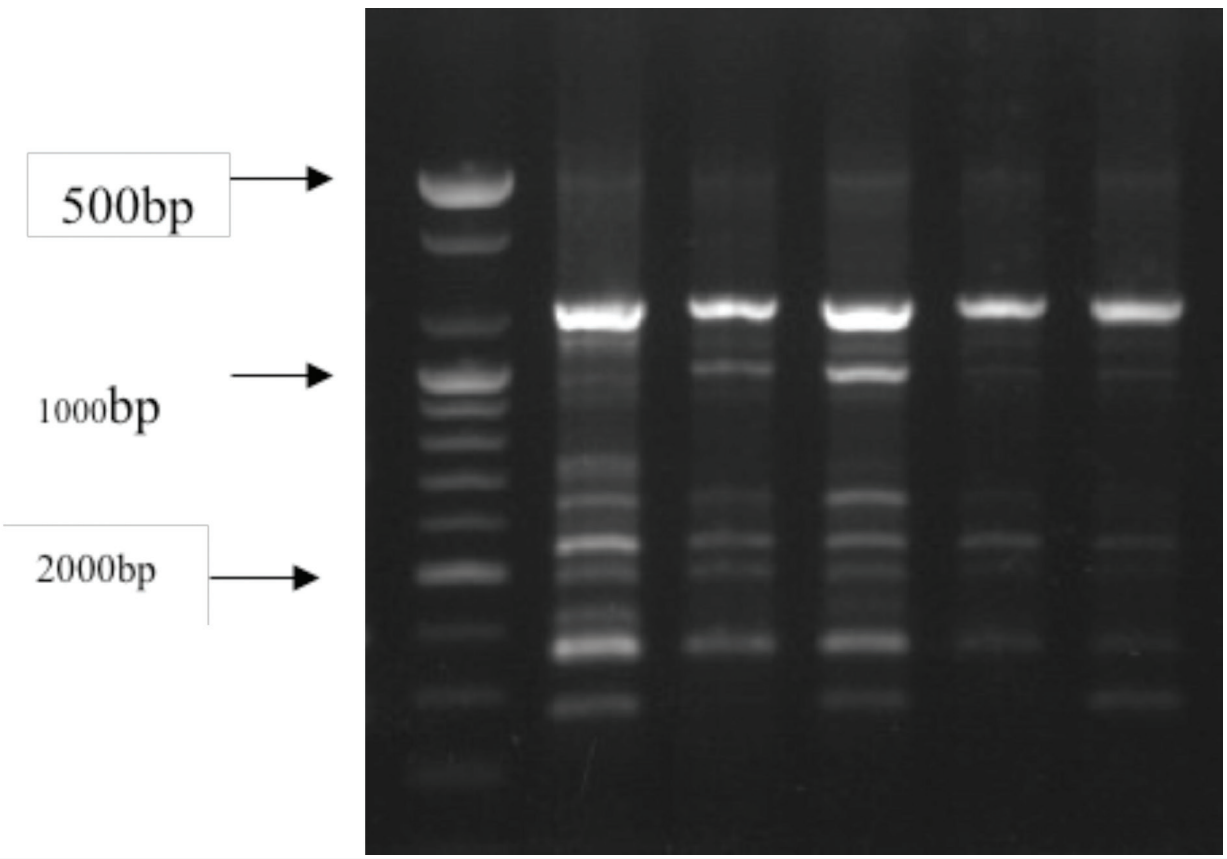

Figure 1. Agarose gel electrophoresis of RAPD fragments generated by primer OPS12 of different date palm female cultivars, Molecular marker (bp) (lane 1); Breem cv. (lane 2); Khidrawi Mandli cv. (lane 3); Maktoum cv. (lane 4); Teberzal cv. (lane 5); Osta Emran cv. (lane 6).

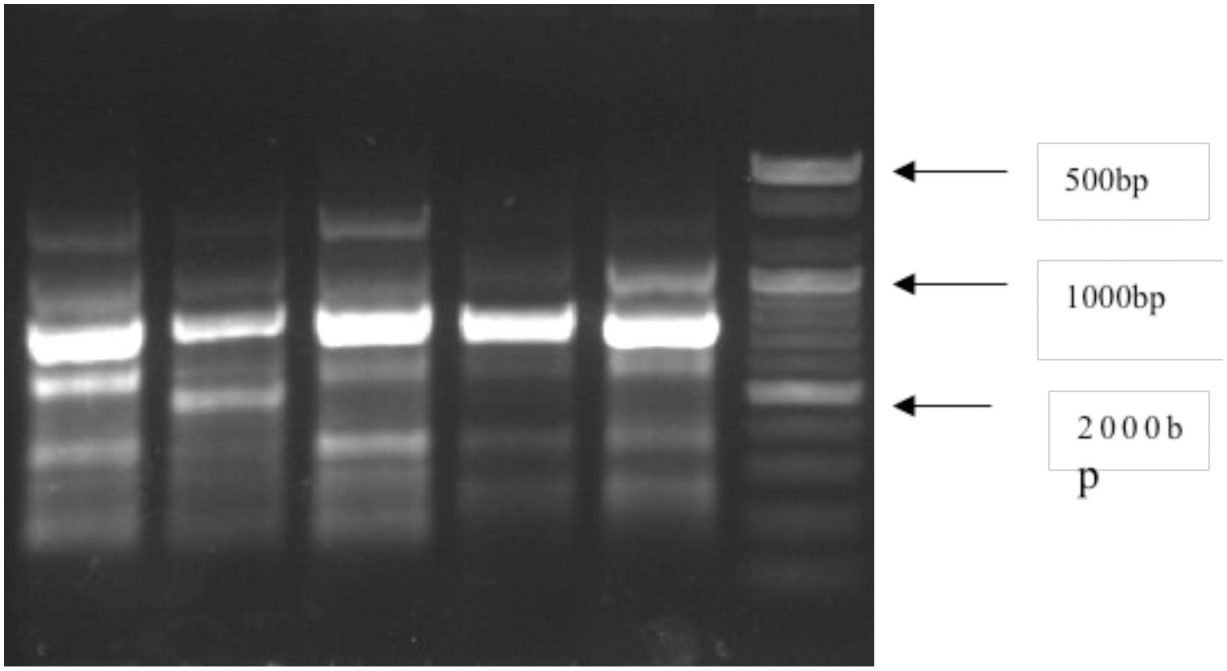

Figure 2. Agarose gel electrophoresis of RAPD fragments generated by primer OPA11 of different date palm female cultivars, Breem cv. (lane 1); Khidrawi Mandli cv. (lane 2); Maktoum cv. (lane 3); Teberzal cv. (lane 4); Osta Emran cv. (lane 5); Molecular marker (bp) (lane 6).

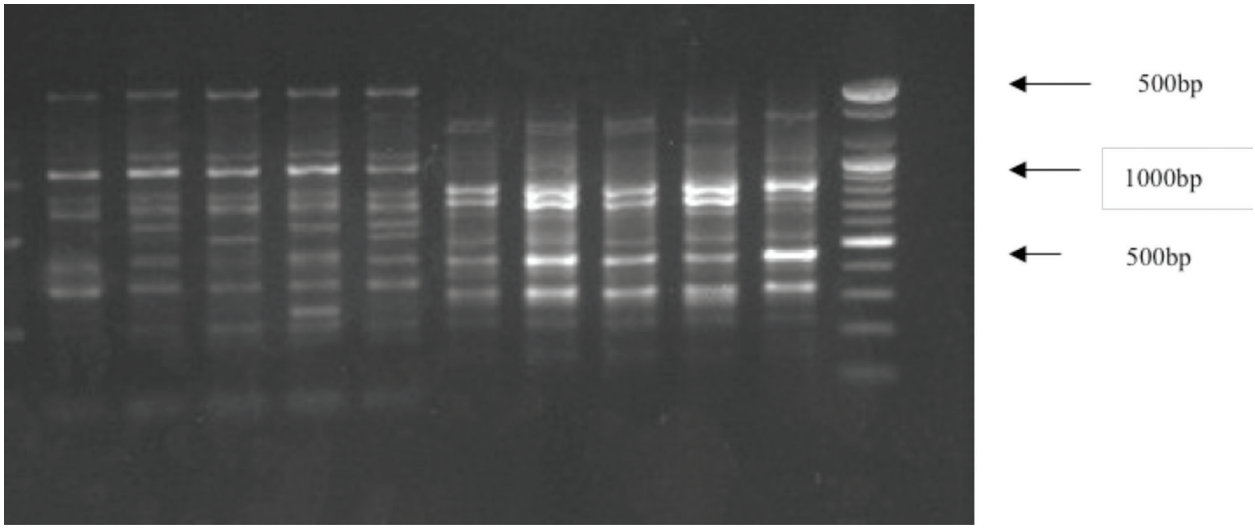

Figure 3. Agarose gel electrophoresis of RAPD fragments generated by primers OPA17 (lane 1-5) and OPC15 (lane 6-10 of different date palm female cultivars, Breem cv. (lane 1); Khidrawi Mandli cv. (lane 2); Maktoum cv. (lane 3); Teberzal cv. (lane 4); Osta Emran cv. (lane 5); Breem cv. (lane 6); Khidrawi Mandli cv. (lane 7); Maktoum cv. (lane 8); Teberzal cv. (lane 9); Osta Emran cv. (lane 10);Molecular marker (bp) (lane 11).

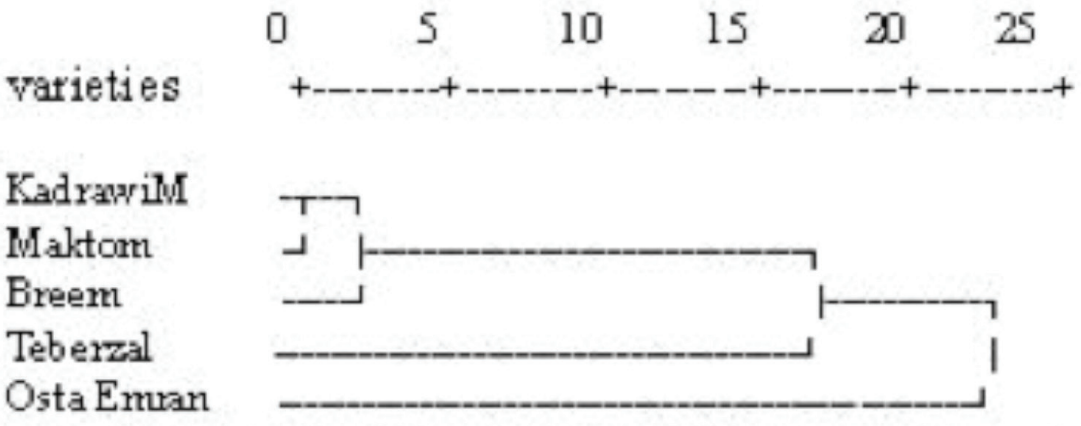

Figure 4: Dendrogram of 5 Iraq date palm varieties based on jaccard genetic similarity

Comparative analysis of annotated genome of date palm and rice shows proliferation of trehalose biosynthetic genes

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ABSTRACT

Agricultural biotechnology is one of the newly emerging technologies of the 21st century for global food security and to benefit the mankind. In order to improve date palm production efficiency in the arid region, we need to deploy favorable strategies to counteract major environmental stresses, such as salinity and drought. Of the several strategies available, use of transgenic approaches and functional genomic tools, probably hold the most promise toward augmenting its production. Now, we can take the advantage of interdisciplinary research approach to confer high levels of tolerance to different abiotic stress in date palm. Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute and in the stabilization of biological structures under abiotic stress in bacteria, fungi and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants", trehalose does not accumulate to significant levels in the vast majority of plants. The recent discovery of the genes that encode trehalose metabolism enzymes in higher plants, and its potential role in modulating carbon metabolism and stress protection, offers new opportunities and challenges for researchers in this field. The specific objective of this study is to perform comparative analysis of trehalose biosynthesis related genes in date palm and rice. Our results from the data on phylogenetic analyses of protein sequences derived from the corresponding DNA sequences from the annotated genomes of a date palm and the rice (both indica and japonica type) plant species suggests the presence of gene families for both trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), indicating the genomic complexity of trehalose biosynthetic genes in plants.

INTRODUCTION

Trehalose [α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside], a dimer of glucose, is present in diverse organisms such as bacteria, fungi, insects, and some invertebrates, and known to have various functions that distinguish it from another non-reducing sugar sucrose [α-D-glucopyranosyl-(1→4)-β-D-fructofuranoside]. There is considerable evidence for a role of trehalose in protection from desiccation, salinity, osmotic stress as well as extreme temperatures by stabilizing dehydrated enzymes, proteins, and lipid membranes efficiently, in addition to protection of biological structures from damage against a variety of environmental stresses (Crowe *et al*. 1992, Crowe 2007). At the same time, details of both the physiological functions and regulation of the trehalose biosynthetic pathway remain largely unknown. In bacteria there are five different trehalose biosynthetic routes, whereas in fungi and plants there is only one (Avonce *et al.* 2006). The single pathway for trehalose biosynthesis that is common to both prokaryotes and eukaryotes consists of two reactions. First, trehalose-6-phosphate is generated from UDP-glucose (UDP-Glu)

and glucose-6-phosphate (G6P) in a reaction catalyzed by trehalose-6-phosphate synthase (TPS, EC 2.4.1.15). T6P is then dephosphorylated to form trehalose via trehalose-6-phosphate phosphatase (TPP, EC 3.1.3.12). TPS and TPP genes were functionally identified in *Arabidopsis thaliana* by complementation of yeast mutants (Blazquez *et al*. 1998). Homologous TPS and TPP genes have now been identified in many other plant species. These results suggest that trehalose synthesis may in fact be ubiquitous among angiosperms, although the levels to which it accumulates are generally low (Goddijn and Van Dun 1999).

MATERIALS AND METHODS

The present study was carried out using the tools of bioinformatics and computational biology. NCBI/TIGR genome data bank (for rice genomic DNA sequencing data) and Weill Cornell Medical College in Qatar date palm sequencing project data was used for our phylogenetic analyses. The rice (*Oryza sativa* L.) genome sequences of the *indica* cultivar (I) 93-11 and *japonica* cultivar (J) Nipponbare and date palm (*Phoenix dactylifera L.*) cultivar Khalas were searched with the BLASTN algorithm for genes with similarity to the TPS = Trehalose-6-phosphate synthase, TPP = Trehalose-6-phosphate phosphatase, and TRE = trehalase genes of *Arabidopsis thaliana* L. ecotype Columbia. Locus names may be accessed at NCBI GenBank database.

RESULTS AND DISCUSSION

Comparative analysis of the genomic sequences of two plant species [*Oryza sativa* (both *indica* and *japonica* cultivars) and *Phoenix dactylifera*] suggests a proliferation of putative genes encoding TPS and TPP enzymes (Table 1). Altogether, there are 11 and 11 putative TPS-like proteins, and 11 and 7 putative TPP-like proteins within the respective genomes. The TPS gene family clusters into two distinct groups, the class I subfamily of TPS genes encodes catalytically active TPS enzymes, whereas the class II TPS genes encode inactive TPS-like proteins with a C-terminal TPP-like domain (Leyman *et al*. 2001, Lunn 2007). In general, the class II genes contain two phosphatase consensus sequence boxes that have been found in all class III TPP genes. In contrast, several of the class I genes from rice and date palm does not contain the phosphatase-specific part in the C-terminal region. Thus, these representative genes probably all may contain only TPS enzyme activity. Amino acid identity between the members of the class I and class II genes is approximately 30-40%. Based on the amino acid sequence similarity, less consistency in tree topology was found in class II TPS, and class III TPP gene families compared to class I TPS genes (Data not shown). The class IV trehalase gene family is much smaller, and often represented by a single gene, and most closely related to those from animals, indicating a eukaryotic origin of this gene. So far, only TPS1 gene from a few plants are known to encode an enzymatically active TPS, which is able to complement the yeast *tps1*Δ mutant and showed restoration of trehalose synthesis and growth on glucose (Blazquez *et al*. 1998, Van Dijck *et al*. 2002). In contrast, class II AtTPS7 or AtTPS8 were unable to complement the yeast *tps1*Δ mutant (Vogel *et al*. 2001), and AtTPS5 shows no TPS activity (Harthill *et al*. 2006). Thus, whether the class II proteins actually have TPS and/or TPP activities remains unresolved, and suggests that there may be fundamental differences in the properties and/or functions of the two distinct subfamilies of TPS genes (Lunn *et al*. 2006).

Moreover, the complete genome sequencing of *Arabidopsis thaliana* and *Oryza sativa* has revealed complex genomic organization of plant trehalose biosynthesis genes (Leyman *et al*. 2001, Lunn 2007, Ramon and Rolland 2007). The Class I (TPS1-4) and Class II (TPS5-11) are most similar to the *E.coli otsA*, except that the catalytic activity of TPS enzymes has not yet unequivocally demonstrated for the latter group (Table1). Class III (TPPA-TPPK) contains a family of smaller proteins similar to the *E.coli otsB* with two conserved phosphatase box. Both rice and date palm contain a single gene encoding for trehalase enzyme (Table1). Based on the comparison of the protein sequences, we found five highly conserved regions in most of the proteins of TPS and TPP in date palm and rice. Also, we found a high degree of conservation of active site residues in the three conserved regions of TPP proteins.

Recently, several research groups have reported on genetic manipulation of trehalose biosynthetic genes in plants and its impact on agronomic traits (Garg *et al.* 2002; Jang *et al.* 2003; Miranda *et al.* 2007; Garg *et al.* 2013). Although these phenotypes indicate that trehalose affects many aspects of metabolism, growth and development, nevertheless, it is difficult to distinguish which of the changes are direct, and which are indirect. Recently, the expression pattern of the 11 AtTPS genes in *Arabidopsis* shows that they are expressed in a developmentally programmed and tissue-specific manner, implying a relevant function in cell metabolism (Avonce *et al*. 2006).

In conclusion, the recent discovery of a plethora of genes that encode trehalose metabolism enzymes in higher plants, and its potential role in modulating photosynthesis, carbon metabolism and stress protection, has led to a series of scientific surprises and offers new challenges for researchers in this field. In view of the latest findings, trehalose research in plants should be seen as an opportunity to use multidisciplinary approaches for the dissection of metabolic networks, including the interface between sugar sensing-signaling and carbohydrate metabolism.

Acknowledgments

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Table

Table 1: The gene families found for trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) genes from the completely sequenced genomes of *indica* and *japonica* rice, as well as *Phoenix dactylifera*.

Oryza sativa			Phoenix dactylifera	
Indica		Japonica		
Class	Protein name	Locus name	Protein name	Protein name
Class I (TPS)	OsI-TPS1 OsI-TPS2 OsI-TPS3 OsI-TPS4	EAY98715 EAZ09170 EAZ07161 EAY87814	OsJ-TPS1 OsJ-TPS2 OsJ-TPS3 OsJ-TPS4	Pd-TPS1 Pd-TPS2 Pd-TPS3 Pd-TPS4
Class II (TPS/TPP)	OsI-TPS5 OsI-TPS6 OsI-TPS7 OsI-TPS8 OsI-TPS9 OsI-TPS10 OsI-TPS11	EAY89092 EAZ09017 EAY75710 EAY75823 EAY98705 EAZ06991 EAZ08891	OsJ-TPS5 OsJ-TPS6 OsJ-TPS7 OsJ-TPS8 OsJ-TPS9 OsJ-TPS10 OsJ-TPS11	Pd-TPS5 Pd-TPS6 Pd-TPS7 Pd-TPS8 Pd-TPS9 Pd-TPS10 Pd-TPS11
Class III (TPP)	OsI-TPPA OsI-TPPB OsI-TPPC OsI-TPPD OsI-TPPE OsI-TPPF OsI-TPPG OsI-TPPH OsI-TPPI OsI-TPPJ OsI-TPPK	EAY79464 EAZ03880 EAY86968 EAY95105 EAY76459 EAZ00197 EAZ06967 EAZ08844 EAY90273 EAZ04762 EAY82521	OsJ-TPPA OsJ-TPPB OsJ-TPPC OsJ-TPPD OsJ-TPPE OsJ-TPPF OsJ-TPPG OsJ-TPPH OsJ-TPPI OsJ-TPPJ OsJ-TPPK	Pd-TPPA Pd-TPPB Pd-TPPC Pd-TPPD Pd-TPPE Pd-TPPF Pd-TPPG
Trehalase	OsI-TRE1	EAY79237	OsJ-TRE1	Pd-TRE1

Identification of cost effective plantlet regeneration method for commercial-scale date palm micropropagation

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ABSTRACT

An investigation on multiple plantlet regeneration methods from date palm shoot tip and leaf explants was carried out at the Plant tissue culture unit of the Environment and Life Sciences Research Centre of Kuwait Institute for Scientific Research to identify the most cost effective method of plantlet regeneration for date palm micropropagation. Four cultivars namely Barhi, Madjhool, Khlas and Suckari were used for the experimental study. The media and protocol developed for the date palm micropropagation at KISR were used for the regeneration experiments. Eight different in vitro methods of plantlet regeneration were developed during the study. Each method had its own merits and demerits. Plantlets were produced through each method of in vitro regeneration method, hardened and planted in the field for evaluation. Plants produced through all the 8 different methods of regeneration were confirmed true-to-type in the field. Economic feasibility study was conducted for each method of regeneration. Among all the eight different methods of plant regeneration, direct somatic embryogenesis method of regeneration was identified as the most cost effective method of regeneration for the commercial-scale date palm micropropagation.

Keywords: Tissue culture, somatic embryogenesis, organogenesis, acclimatization, *in vitro*.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is propagated traditionally by offshoots or suckers, which are produced in the leaf axils and usually appear at or below the ground level surrounding the stem base. Small offshoots that appear above the ground level on the trunk are usually destroyed due to difficulty in rooting. Offshoots are produced in a limited number for a certain period in the lifetime of a young date palm tree. Offshoot formation is dependent on the genetic makeup of the cultivar and the environmental factors. The number of offshoots produced by an individual date palm tree is highly variable and varies cultivar to another. The traditional method of vegetative propagation through offshoot is slow, laborious, time-consuming and expensive. Transmission of disease-causing pathogens and insects is another disadvantage of conventional offshoot propagation. This has focused on micropropagation technology development during the past 40 years for rapid clonal reproduction of selected cultivars which is cheaper than the offshoot method.

Date palm tissue culture was initiated with little success in the year 1970 (Schroeder, 1970) and succeeded in developing protocols during late 1970s (Ammar and Benbadis, 1977; Tisserat, 1979). Free-living date palm plantlets using tissue culture method was succeeded during 1980s using shoot tip tissue explants (Tisserat and DeMason, 1980; Tisserat, 1981; Beauchesne, 1982; Zaid and Tisserat, 1983). The culture media and protocol was either adopted or modified and used for the micropropagation of date palm worldwide later on (Sharma et al, 1986; Sudhersan, 1989). Date palm micropropagation using immature flower buds was reported in 1985 (Drira and Benbadis, 1985). Several date palm cultivars have been micropropagated in various laboratories in different parts of the world. Organogenesis and somatic embryogenesis are the common methods of regeneration practiced for this purpose (Beauchesne, 1982; Reuveni, 1979; Sharma *et al.,* 1986; Sudhersan *et*

al., 1993). Literature study on date palm micropropagation indicated that complicated culture media with different combinations of growth hormones and organic additives were reported for plantlet regeneration. In the present study a simple and efficient protocol was established to regenerate plantlets through multiple regeneration methods. Generally occurring tissue browning, *in vitro* plantlet dwarfing and hyperhydricity were also controlled through this simple protocol. Plantlets produced by all this methods were proved to be clonal in nature (Sudhersan and AboEl-Nil. 2004). The details of the study are presented in this report.

MATERIALS AND METHODS

Plant Material

Offshoots of date palm cultivars Barhi, Khlas, Madjhool and Succary were used as plant material. All the offshoots were collected from the date palm orchard maintained inside the Kuwait Institute for Scientific Research (KISR) campus, Shuwaik, Kuwait.

Explant Preparation

The older leaves of the offshoots were dissected out acropetally and the shoot tip with few young leaf primordia were isolated and surface sterilized prior to explant preparation. Surface sterilization was carried out using 100% commercial Chlorox with a drop of Tween 20 for 30 min followed by thorough washing in sterile distilled water for five to six times. All surface sterilization activities were carried out under the laminar hood. The explants were isolated using a sterile forceps and scalpel with surgical blade under aseptic conditions. The explants (whole leaf primordia with leaf sheath, shoot meristem with stem tissue and irregularly cut shoot tip tissue) were prepared for the regeneration experiments. Two offshoots from each cultivar were used for the study.

Culture Medium

The culture medium used by Tisseret (1979) was modified and formulated a five stage culture media: 1. initiation, 2. regeneration, 3. growth and multiplication, 4. elongation and rooting and 5. photoautotrophic culture for this study. The pH of the media was adjusted to 5.6 using 1 N NaOH or 1 N HCl before adding the agar. All media were sterilized by autoclaving at 121° C and 101Kpa for 15 min.

Culture Initiation and Incubation

Two sets of explants prepared from each cultivar were inoculated aseptically on to the initiation media. One set of cultures were maintained under total darkness at 25º C temperature and another set was maintained at 16 h light and 8 h dark culture condition. Cultures maintained at total darkness was subcultured in the same media once in 30 days and cultures maintained at light were subcultured once in a week. After 60 days all the explants were transferred to the regeneration media.

Regeneration

After transferring to the regeneration media, 50% of the cultures including the shoot meristem maintained at total darkness were transferred to 16 h light and 8 h dark culture condition and the remaining 50% were maintained at total darkness. All cultures were subcultured once in 15 days regularly in the same regeneration media. Observations were made regularly once per week on callusing and direct regenerations on the tissue explants. All types of regeneration methods were photographed and recorded.

Growth and Multiplication

Tissue explants or leaf primordial expalnts showing embryo or shoot bud were transferred to the growth and multiplication media and subcultured once in every 15 days regularly. Somatic embryos or shoot buds were isolated and maintained in the same media during each subculture. Germinated plantlets were isolated and subcultured in elongation and rooting media.

Elongation and Rooting

Germinated plantlets were isolated and transferred to the elongation and rooting media and maintained for 20-30 days. After 30 days all the rooted plantlets were removed for photoautotrophic culture and the remaining un-rooted plantlets were subcultured in the same rooting medium for rooting.

Photoautotrophic Culture

Photoautotrophic culture media was prepared by mixing quarter strength MS macro and micro nutrients (Murashige and Skoog, 1962) with a soil mix prepared by mixing peat moss and garden soil at 1: 1 ratio and autoclaved at 121˚ C for 30 min and cooled at room temperature. Rooted plantlets were washed in soap solution and rinsed in sterile water for three times and planted in plastic trays containing 100 planting cells of 5cm side and 10cm height. All plantlets were sprayed with 0.5 g/l solution of fungicide Topsin® and kept inside the poly carbonate transparent culture box in closed condition. Photoautotrophic culture boxes were maintained inside the growth rooms at 25±2˚C, 3000 lux light intensity and 12 h photoperiod. After 30 days all boxes were transferred to the greenhouse for acclimatization.

Acclimatization

All the plantlets were maintained inside the temperature and humidity controlled greenhouse for a week time and the boxes were kept opened for 4 h daily for

another week and the plantlets were exposed to the greenhouse conditions completely afterwards. All plantlets were transferred to same soil media in larger pots and maintained in the nursery benches for 30 days to grow and later on transferred to 1gallon plastic pots and maintained in the shade-house for field transfer.

RESULTS

Effect of light

The cultures maintained at total darkness did not show any browning. All cultures kept under light started browning. Browning of tissues maintained at light was controlled by subculture frequently at 7 d interval. Greening and tissue maturation were observed in expalnts maintained at light. All tissue explants turned to light yellowish in color under total dark condition. Callusing was delayed in light and callusing was faster under dark. Direct somatic embryos and shoot buds were regenerated faster under light and slow under total darkness. Percentage of regenerating tissue explants was more in dark and less under light.

Effect of Explants

Among the whole leaf primordia, shoot meristem and irregularly cut shoot tip tissue, irregularly cut tissues callused faster and produced callus within 60 days under total darkness. The whole leaf primordial explants produced callus only at the basal sheath region and at the laminal region. Shoot meristem enlarged and did not produce callus within 60 days period. Globular meristomoids were observed on the cut end of the tissues initially prior to callus initiation under dark condition. Under prolonged dark condition, the meristemoids turned in to callus in the initiation media. When transferred to the regeneration media and maintained at light, the meristomoids produced somatic embryos or shoot buds. The shoot meristem gradually turned into green and elongated into plantlet within 90 d. The irregularly cut tissue explants transferred in to the regeneration media and maintained under light produced somatic embryos and shoot buds directly or through callus. There were three types of calli: 1. mucilaginous, 2. nodular and 3. spongy observed on the cut tissue explants. The whole leaf explants showed direct regeneration.

Types of Regeneration

After 30 days under darkness in the initiation media, the explants prepared through irregular cuts showed induction of globular meristemoids. After another 60 days under the darkness in the same initiation media under total darkness, calli were proliferated and three types of calli were recognized: 1. nodular, 2. spongy and 3. mucilaginous. When transferred to the regeneration media under light, nodular callus differentiated into green organogenic and yellowish embryogenic callus. The mucilage callus produced only yellowish embryogenic callus. The whole leaf primordial explants produced meristomoids at the basal sheath area and laminal tissue area in the initiation media. When the leaf primordial explants transferred to the regeneration media and maintained under light, produced somatic embryos and shoot buds directly. The shoot meristem explant when transferred to the regeneration media and maintained at light turned in to green and elongated in to plantlet with compound leaf. Axillary shoots were appeared from the leaf axils. A total of 8 types of plantlet regeneration: 1. Direct somatic embryogenesis, 2. Indirect somatic embryogenesis, 3. Direct shoot induction, 4. Indirect shoot induction, 5. Axillary shoot induction, 6. Adventitious shoot formation from the shoot buds, 7. Direct somatic embryogenesis from shoot buds and 8. Direct plant regeneration from meristem (Figs. 1-9).

Plantlet Regeneration

Plantlets were produced in growth and multiplication media through all the 8 different methods. The somatic embryos multiplied by secondary somatic embryogenesis and germinated in to plantlets. Shoot buds produced plantlets directly or through somatic embryos produced at the basal region of the shoot buds. Shoot meristem produced axillary shoots and plantlet. More number of plantlets were produced through somatic embryos obtained through any method of regeneration. Plantlets were produced continuously using the multiplication and growth culture media.

Photoautotrophic Culture

In the photoautotrophic culture media, plantlets were more hardened and the roots were multiplied. More capillary roots were also induced. The leaves turned dark green in colour. The surface of the leaves produced more waxy coating on the surface during the photoautotrophic culture phase.

Acclimatization

Plantlets produced through all the regeneration methods were hardened through two methods: 1. direct transfer from the rooting stage and 2. transfer from the photoautotrophic culture. Plantlet survival was 50% by the first method and 100% through the second method. Plantlets acclimatized through photoautotrophic method showed faster growth.

Field Evaluation

Plantlets produced through all the 8 types of regeneration method were planted in the date palm orchard inside the KISR campus and maintained. All the palms produced by all the eight different methods showed faster vegetative growth, produced axillary shoots during the vegetative growth phase and started producing flowers during the 4th year of planting. Palms produced by all the eight different methods were found to be true to type.

Feasibility

The minimum cost for producing 25,000 plants through each method was calculated. among all the eight methods studied, the direct somatic embryogenesis method was the most efficient and highly economically feasible method of plant production.

DISCUSSION

Date palm micropropagation research started during1970s (Hoded, 1977; Reuveni *et al.,* 1974; Tisserat *et al.,* 1979). Immature or mature somatic embryos were cultured on MS culture media containing many organic additives, an auxin (2,4-D) and a cytokinin (2iP) or kinetin (K) for callusing and somatic embryogenesis. During the latter stages, somatic embryogenic calli were obtained from tissue explants from the shoot tip and plantlets were regenerated and planted in the year 1982. Starting from 1982 many laboratories carried out research and development on date palm micropropagation. Literature study indicated different types of regenerations, complicated culture media formulations and physiological disorders like tissue browning, hyperhydricity and dwarfing which affected plant production (Zaid and De Wet, 2002). However, many laboratories developed their own techniques for the date palm micropropagation through somatic embryogenesis or organogenesis.

Through 15 years of research and development on date palm micropropagation, we have developed a five stages of culture media through modifications of the culture media reported by Tisseret (1979). Through this five different stages of culture media we were able to produce large number of plantlets without any genetic disorders. Techniques were also developed for controlling tissue browning and dwarfing of *in vitro* plantlets without any changes in media components. This media was successfully used for the micropropagation of 40 different date palm cultivars in our laboratory.

Generally according to the published reports, the shoot tip used to be cut irregularly into small pieces and used as explants for somatic embryogenesis, and leaf primordial explants were used for organogenesis. The initiation media used to have the growth hormone 2,4-D for somatic embryogenesis and auxins other than 2,4-D and cytokinins for organogenesis. Under total darkness the tissue explants produce callus after 3-6 months and later the nodular callus produces somatic embryos in the case of somatic embryogenesis method and shoots were inducted from the leaf base in the case of organogenesis. In our study, we have observed multiple regeneration method using a single culture initiation media through manipulation of explants and timing on transfer from initiation media to regeneration media.

Initially, tissue explants of date palm in the presence of 2,4-D produces globular structures called meristemoids at the cut ends of the tissue explants after 30 d of culture. On prolonged culturing in the same media, the meristemoids grow further and produces different types of callus. If the explants with meristemoids are transferred to the regeneration media and maintained under light, the meristemoids turned to develop into shoot bud or somatic embryos directly. From this it is clear that the growth hormone 2,4-D induces the regeneration potential of the explants and supported callusing afterwards. The leaf margins, leaf base, leaf sheath rachis and petioles are potential in regeneration.

The maintenance of the explants with 2,4-D induced meristemoids (proembryos) on the same media for 90 d induce somatic embryogenic callus. On transferring these somatic embryogenic callus to the maturation media produces somatic embryos followed by secondary somatic embryos. The direct somatic embryogenesis and indirect somatic embryogenesis occurred in the same culture medium and the culture time factor controls the two types of embryo production. Removal of somatic proembryos from the 2,4-D medium at the right time and subculture to maturation media avoids the somatic embryogenic callus formation. Secondary somatic embryo formation was found to be a continuous process which occurred in PGR-free MS culture media for several years when the cultures were subcultured periodically once in 15 d. The mature somatic embryos germinated into plantlets with a single thin tap root. On trimming the taproot and transferring the plantlets to a rooting media containing produces several healthy adventitious roots after 20-30 d in culture. One hundred percent plantlets produced by our protocol survived during acclimatization. However, less than 5% plant mortality was observed during the long term nursery maintenance.

Our study concluded that the auxin 2,4-D is mainly necessary for any type of regeneration at the culture initiation stage. Timing and explant type are the important factors which decides the type of regeneration. Among all the 8 different types of regeneration methods, direct or indirect somatic embryogenesis is preferred to commercial production. All other methods can be used for the experimental purpose. Organogenesis is a slow method which takes 12 -24 months for the initial establishment. However, after producing the adventitious plantlet multiplication stage, plant production will be faster and similar to the somatic embryogenesis method. Through the rejuvenation method, very old trees of commercially important characters can be rejuvenated in to young plants. Our protocol works out for clonal propagation of any date palm cultivar. Among all the possible methods of plant production, direct somatic embryogenesis method was found to be highly economically feasible method.

Acknowledgement

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Figures:

Figures1-8: Date palm *in vitro* regeneration types.


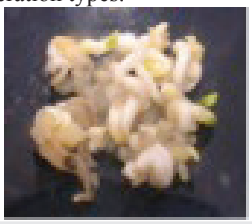

Fig. 1. Direct somatic embryogenesis; Fig. 2. Indirect somatic embryogenesis; Fig.3. Direct organogenesis; Fig. 4. Indirect organogenesis;

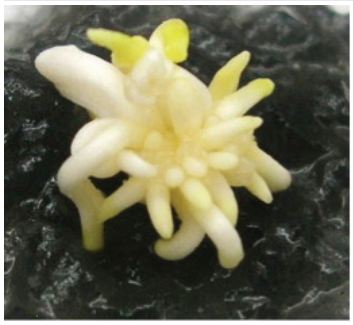
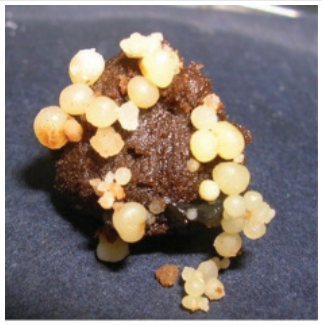
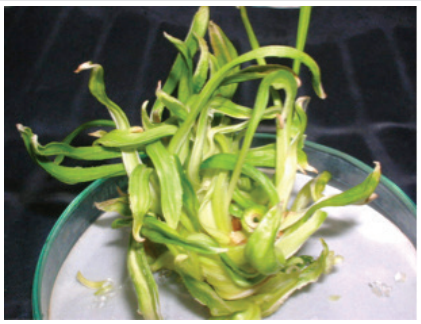


Fig. 5. Somatic embryogenesis from shoot bud; Fig. 6. Meristemoids regeneration; Fig. 7. Axillary shoot induction; Fig. 8. Plant regeneration from shoot meristem.

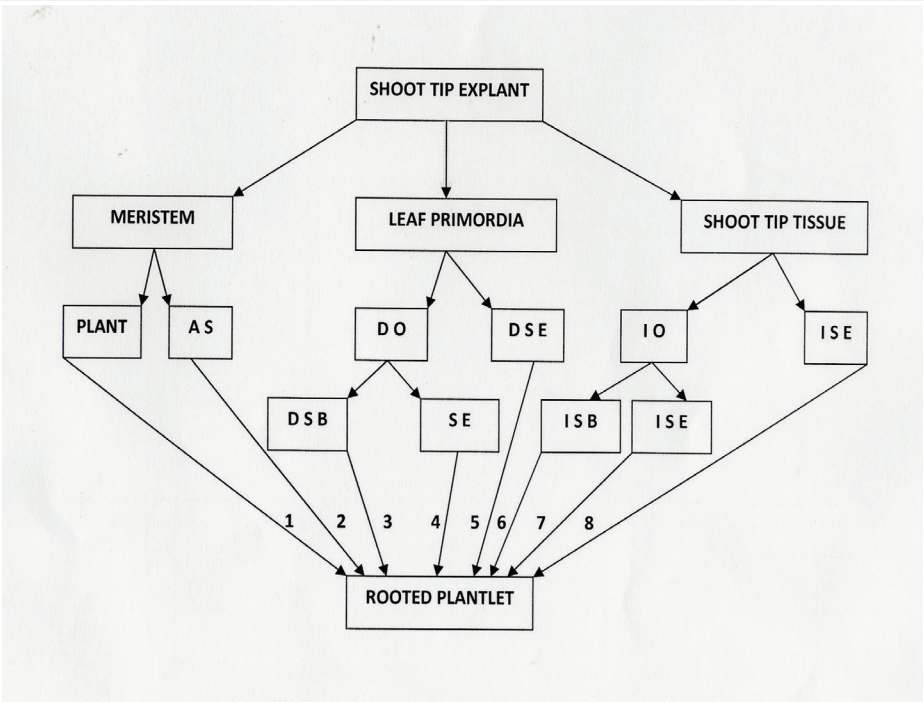

Fig. 9. Different regeneration methods of plantlet regeneration in date palm. AS-Axillary shoot; DO-Direct organogenesis; DSE-Direct somatic embryogenesis; IO-Indirect organogenesis; ISE-Indirect somatic embryogenesis; DSB-Direct shoot bud; SE-Somatic Embryogenesis; ISB-Indirect shoot bud.

Somatic embryogenesis in *Phoenix dactylifera*: maturation, germination and reduction of hyperhydricity during embryogenic cell suspension culture

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ABSTRACT

This work describes an improved and efficient method for optimum maturation and normal embryo growth to produce healthy plantlets through somatic embryogenesis using suspension cultures for date palm cv. Sakkoty. Cell suspensions were supplemented with low amounts of dichlorophenoxyacetic acid (0.5mg/1) and different concentrations of abscise acid (ABA) (0.1, 0.3 and 0.5 mg/l) and polyethylene glycol (PEG 4000) (1, 3 and 5g/l). Factors affecting embryogenic callus maturation and germination embryos were investigated. Somatic embryos and callus fresh weight increased at 3g/l of PEG 4000. Treated somatic embryos with 5g/l PEG reduced hyperhydricity. Transferring them on the germination medium (G1) supplied with 3 g/l PEG improved the embryo growth. Secondary embryos were produced at 0.5 mg/l ABA .Shoot proliferation and development of plantlets occurred on medium containing 0.1 NAA mg/l, 0.2 mg/l BA and 0.2 mg/l kin (G2). The highest accumulation of proteins was obtained with 0.5 or 0.3 mg/l ABA. The plantlets were transferred successfully to greenhouse.

Keywords: *In vitro,* Date palm, ABA, PEG, Somatic embryogenesis, Maturation, Desiccation, Germination and proteins.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world and is mentioned in the Holy Qur'an and Bible. Date palm is one of the most important fruit trees in the Middle East and in the Saharan and Sub-Saharan regions of Africa. In some areas, this is the only tree which provides food, shelter and fuel to the communities. Dates are not only a staple food but are also an important export cash crop (Zaid and Hegarty, 2006). Embryogenic suspension culture is defined as single cells or small cell aggregates in agitated liquid media (Preil, 2005).

The use of large-scale liquid cultures and automation have been well documented, and benefit have been shown both for resolving the manual handling of various stages of micropropagation, decreasing production cost signiafantly and for better plant performance by allowing a direct contact of the medium throughout the plant material (Zobayed and Saxena, 2003).

A typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with callus induction, embryogenesis callus multiplication, somatic embryo maturation and somatic embryo germination (El Hadrami, 1995 and El Bellaj, 2000). In most cases, embryogenic calluses were induced on medium containing growth regulators, especially 2,4-D (El Hadrami, 1995; ELBellaj, 2000; Fki *et al.,* 2003 and Gadalla, 2007)). Maturity of somatic embryos may be induced by the application of exogenous ABA (El Bellaj, 2000 and Corredoira *et al.,* 2003).

Label and Lelu (2000) indicated that ABA plays an important role in both somatic and zygotic embryo maturation. These same authors indicated that ABA promotes embryo maturation, supports the accumulation of storage proteins, lipids and starch; it suppresses the formation of aberrant embryo structures and, finally prevents the mature embryo from germinating precociously. Choi *et al*, (1999); Kim *et al.,* (1999) and Klimaszewska *et al.,* (2001) reported that the culture medium constituents particularly osmoticum, has a marked effect on somatic embryos. Also, the attempt to increase the quality of somatic embryos by using the high molecular mass osmoticum, PEG 4000, and ABA was accomplished by insertion of a maturation phase of culture between multiplication (maintenance) and regeneration phase. The combined application of ABA and PEG has become a routine method for stimulation of somatic embryo maturation in some gerera of coniferales (Bozhkov and Von Arnold 1998) and selected tree species such as *H. braziliensis* (Linossier *et al.,* 1997).

This paper describes an improved and efficient method for optimum maturation and normal growth of embryo to produce healthy plantlets without hyperhydricity through somatic embryogenesis using suspension cultures for date palm cv. Sakkoty; the most common dry cultivar in Upper Egypt.

MATERIAL AND METHODS

Plant material and culture conditions

Embryogenic cultures were induced from shoot tips of *Phoenix dactylifer*e L. (cv.Sakkoty)cultured on solid medium containing MS salt and vitamins (Murashige and Skoog, 1962), 10mg/l 2,4-D, 3mg/l 2ip, 40 g/l sucrose, 200 mg/l glutamine, 40 mg/l adenine sulfate and 1.5 g/l activated charcoal (AC) solidified with 6 g/l Agar-Agar. Prepared medium was adjusted to pH 5.7 ± 0.1 and distributed into small jars (200 ml), each one contains 50ml of prepared medium and then autoclaved at 121 °C and 1.5 cm /ins2 for 20 min. Cultures were kept in darkness at 28 ± 2 °C and re-cultured every 6 weeks until the initiation of embryogenic callus.

Establishment of embryogenic suspensions

Five hundred milligrams of friable callus was chopped into small pieces and transferred aseptically into 50 ml liquid medium in 250 ml Erlenmeyer flasks containing half salts and vitamins (Murashige and Skoog, 1962) except Fe-EDTA which was full strength, 0.5 mg/l 2.4 –D, 200 mg/l KH2PO4, 40 gm sucrose, 100 mg/l myoinsitol and 100 mg/l Arginin, 100 mg-1 glutamine, 0.3 g activated charcoal (AC) (Gadalla, 2007), in addition to different concentrations of ABA (0.1, 0.3 and 0.5 mg/l), polyethylene glycol 4000 (1,3 and 5 g/l) . Cultures were maintained on a rotary shaker at 120 rpm at 25 ± 2 °C under darkness. Suspensions were subcultured every two weeks by decanting off the old medium and replacing it with fresh medium of the same composition for maturation. Embryogenic cell clumps were filtered through a 1 mm sieve to determine number of somatic embryos and callus fresh weight.

Partial desiccation and reducing Hyperhydricty

The developed of embryos occurred after sieving (mesh size= 1 mm) were put in sterile empty Petri dishes containing two sterile Whatman filter paper disks and kept in the dark for 2 h desiccation. The partial desiccation embryos were transferred to solid medium. After desiccation, three somatic embryos / jar from every concentration of ABA and PEG was cultured on MS solid medium supplemented with the same composition, each treatment consisted of three replicate . Data were taken on the hyperhydricy percentage (vitrified embryos/ total embryos*100), number of secondary embryos and germination percentage (G1) after one subcultures (4 weeks).

Germination and shoot proliferation of somatic embryos

Advanced somatic embryos produced were cultured on MS solid medium supplemented with 0.1 NAA mg/l + 0.2 mg/l BA+0.2 mg/l kin, 200 mg/l KH2PO4, 40 g sucrose, 100 mg/l myoinsitol, 100 mg/l glutamine and 0.3 g AC. The medium was distributed to small jars (200ml), each one contains 40 ml. Cultures were kept in light (2000 lux) at 25 ± 2 °C. Data were taken on the germination percentage (G2) (embryos with single shoot and root / total embryos x 100) and number of shoots after two subcultures (8 weeks). Advanced somatic embryos were considered germinated as soon as radical emergence occurred with plantlet based on shoot greening and elongation.

Rooting stage

Plantlets were cultured on *1/2* MS liquid medium supplemented with 1.0 NAA mg/l, 200 mg/l KH2PO4, 40 g sucrose, 100 mg/l myoinsitol and 1g/AC, and incubated under 6000 lux light (Fig.3c).

Plant acclimatization

Healthy regenerated plantlets were individually removed from tubes, agar was rinsed off, and plants were cultivated in plastic pots filled with peat moss and perlite (1:1 ratio) in a greenhouse (Fig.3d).

Extraction and analysis of soluble proteins

The protein was extracted according to the method described by Lecouteux *et al* (1994). Fresh callus (250 mg) was ground with 2 ml of 0.25 M phosphate buffer (pH 7.2) and centrifuged for 3 min at 7000 rpm.

Supernatant was used as the crude extract. The level of total proteins was measured by spectrophotometry at 595 nm according to Bradford (1976) method.

Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980). LSD at 5% level of significance was used to compare between means according to Steel and Torrie (1980).

RESULTS

Number of somatic embryos and callus fresh weight

Embryogenic callus placed in liquid culture medium with 0.5 mg/ l 2, 4-D in order to examine the influence of ABA and PEG concentration on somatic embryos number and growth of fresh weight Table 1. Both substances were used separately. The highest production of number embryos was obtained with PEG 3g/ l (22.33 embryo/jar), followed by the addition of 0.3 mg/l ABA to culture medium (16.33 embryo/ jar) without significant difference in between while addition of PEG at 5.0 g/l decrease number of embryos significantly (3.66 embryo/jar). Data in the same Table clarified that all concentrations of ABA and PEG added to media increased callus growth. The highest significant value of callus growth was obtained by the addition of 3.0 gm/l PEG (4.413).while the lowest value of callus growth was observed by using PEG at 5.0 gm/l (2.550). However no significant difference could be observed between other treatments under investigation Fig.2.PEG was beneficial to somatic embryo proliferation and increases the quality of mature somatic embryos.

Hyperhydricty percentage, number of secondary embryos and germination percentage (G1)

The duration of the culture period in liquid medium was found to be very important for the balanced germination of somatic embryo, Culture of mature embryos in liquid medium for longer than 1 month led to hyperhydration phenomena (Fki *et al.,* 2003). Interestingly, the partial desiccation of somatic embryos, corresponding to a reduction of hyperhydracty (Fig.1), then transfer embryos to the germination medium (G1) supplied with ABA and PEG concentrations reduced hyperhydricy with more mature somatic embryos formation. The hyperhydricty percentage of somatic embryos (Table 2) was significantly reduced in the presence of PEG; the lowest percentage of hyperhydricty resulted at 5g/l PEG (21.67 %) compared to 0.1mg/l ABA which was 63.33%. The germination rate (G1) of somatic embryos was low when cultured into medium supplemented with ABA and PEG concentration (Fig.3a). The highest germination percentage of somatic embryos was record at 3 g/l and 1 g/l PEG (88.87 and 66.63% respectively) without hyperhydricty compared to the concentration of ABA 0.3 and 0.5 mg/l (11.10%). Data revealed that adding 0.5 mg/l ABA, 3 g/l PEG or 0.3 mg/l ABA to the culture medium was superior in increasing the number of secondary embryos (6.33, 5.66 and 5.33, respectively).

Transferring advanced embryo to medium supplemented with 0.1 NAA mg/l + 0.2 mg/l BA+0.2 mg/l kin proliferated normal shoots. In Table (3), the highest germination percentage (100%) was observed in embryos that had been cultured on media containing 3, 1 g/l PEG or 0.1mg/l ABA. After 8 weeks on proliferation medium, shoot number was determined (Table 3 and Fig.3b). The highest significant value of shoots was obtained with 3g/l PEG (6.33 shoots), followed by 0.1 or 0.3 mg/l ABA resulted in the same value (4.66 shoots). Increasing concentration of ABA or PEG decreased significantly values of shoots (3.00 and 2.66 respectively).

Total protein content

The addition of ABA and PEG had an important effect on date palm protein content. As illustrated in Fig.4, the amount of total proteins increased significantly at 0.5 and 0.3 mg/l ABA (1.591, 1.584 mg/g DW, respectively) followed by 1 g/l PEG (1.504 mg/g DW) in embryogenic callus.

DISCUSSION

Maturation stage

Using 2,4-D at 0.5 mg/l and different concentrations of ABA (0.1,0.3, and 0.5 mg/l) and PEG (1.0,3.0 and 5.0 g/l) enhanced callus fresh weight and number of mature embryos of date palm. These results are in line with those reported by Gadalla (2007) who found that in date palm Khalas cv., liquid culture medium with half strength of MS formula containing 0.5 mg/l 2, 4-D influenced fresh weight of embryogenic callus (5 to 6 fold approximately/month), number of pro-embryo (globular and juvenile) and number of mature embryos). Zouine and El Hadrami (2007) reported that the liquid medium containing 0.1 mg /l of 2,4-D was beneficial for somatic embryo production. Zouine *et al.,* (2005) found that embryogenic callus placed in liquid medium with 10-5 M ABA, protein and sugar accumulation by somatic embryos in liquid culture medium increased linearly as the ABA concentration in the medium (increased from 10-7 to 10-5). Thus, the accomplishment of further maturation stages of date palm somatic embryos seems to be more closely dependent on exogenous ABA. Othmani *et al. (*2009) found that callus transferred to medium supplemented with 1mg/l ABA+ 0.1 g/l AC proliferated normally with an average of 51 somatic embryos observed after about 7 weeks per 0.5 g FW of embryogenic callus. Fki *et al.,* (2003) found that in date palm cultivar (Deglet Nour), the subculture of embryogenic

suspension in a fresh medium with low amounts of 2,4-D (1 mg/l) resulted in the differentiation of a large number of somatic embryos (from 10 to 200 embryos per month per 100 mg fresh weight of embryogenic calli). Fernando and Gamage (2000) concluded the possibility of using ABA to enhance somatic embryogenesis and plant regeneration of coconut. In addition, (Huong *et al.,* 1999) declared that proliferation and maintenance of embryogenic callus of *Phoenix canariensis* was on MS basal medium with 2.26 µM 2,4-D, 0.833µM kinetin and 2 µM abscisic acid (ABA), with a regular subculture every 3-4 weeks. Somatic embryo development was promoted by two months of culture on MS liquid medium enriched with 2µM ABA, for torpedo stage development. (Dunstan *et al.,* 1995) stated that ABA plays an important role in both somatic and zygotic embryo maturation. ABA promotes embryo maturation supports the accumulation of storage proteins, lipids and starch and suppresses the formation of aberrant embryo structures. According to Langhansova *et al*. (2004), a maturation stage was accomplished by insertion of PEG 4000 and ABA between multiplication and regeneration phase. Adding of PEG to maturation medium in many cases has been shown to stimulate maturation (Bozhkov and Von Arnold, 1988).

In our case, the quality of fresh weight was observed in presence of PEG 4000 and number of mature somatic embryos was increased; these data are in agreement with Kong and Yeung, (1995) who found that the number of mature somatic embryos increased significantly when PEG 4000 was applied in maturation medium. The positive effect of osmoticum on embryo maturation has been attributed to increasing levels of endogenous ABA (Wilen *et al*. 1990). Stasolla *et al.,* (2003) reported that, the inclusion of PEG to the culture medium can improve the number and the quality of the embryos produced. Maturation of *Acacia nilotica* (Garg *et al.,* 1996) and *Aesculus hippocasranum* (Capuana and Debergh, 1997) somatic embryos were improved by PEG treatment either alone or in combination with activated charcoal or ABA.

Germination stage

PEG at 5.0 and 3.0 g/l and desiccation treatment caused reduction of hyperhydricty phenomena of date palm. Increasing the concentration of ABA reduced the germination (shoot number) but increased number of secondary embryos. However, using ABA at 0.5, 0.3 mg/l increased protein content. These results are in line with those reported by Zouine *et al. (*2005) reported that the quality of somatic embryos was markedly lowered in the absence of exogenous ABA and a number of hyperhydricty somatic embryos were observed. According to Reidiboym-Talleux *et al*. (1999) hyperhydricty may also be explained by a lack of desiccation period and low endogenous ABA level. Fine chopping and partial desiccation (6 and 12 h) of embryogenic calli with proembryos prior to transfer to MS medium supplemented with 1 mg l−1 ABA stimulated the rapid maturation of somatic embryos. Othmani *et al. (*2009), and Fki *et al*. (2003) reported that, chopping the callus into small pieces favorite the formation of Pro-embryonic masses and the partial desiccation of mature somatic embryos (corresponding to a decrease in water content from 90 to 75%) significantly improved germination rates (from 25 to 80%).

Dunstan *et al*. (1995) stated that ABA prevents the maturing embryos from germinating precociously. Adding of PEG to maturation medium in many cases has been shown to stimulate maturation. There are also reports showing adverse effects of PEG on embryo germination (Bozhkov and Von Arnold 1988). Fernando *et al. (*2003) revealed that abscisic acid induced plant regeneration through somatic embryogenesis of oil palm. Polyethylene glycol 4000 (PEG 4000) was reported to improve germination frequencies (root and shoot emergence) with limiting embryo histodifferentiation in soybean somatic embryo (Walker and Parrott, 2001). Likewise in spruce, it was found that PEG might improve the quality of somatic embryos by promoting normal differentiation of the embryonic shoot and root (Stasolla *et al*. 2003). Sghaier *et a*l. (2009) reported that ABA plays an important regulatory role in ZE maturation and its action was mainly observed on the synthesis of storage proteins by blocking germination and anabolism. This hormone also favors the transport of storage compounds from plant to seeds. A very similar study has been performed in oil palm by Morcillo *et al.* (1999), in which ABA displayed a noticeable role in the accumulation of the total quantity of soluble protein content and especially of the storage protein (Globuline 7S). Similarly, Preeti *et al.* (2004) showed that ABA treatment (5mgl-1) for 14 days significantly increased the starch level and total protein content of *Camellia sinensis* SE. The embryo induction medium supplemented with ABA increased the total storage protein content (to about 29% of embryo DW) in alfalfa SE (Sreedhar and Bewley, 1998). Pliego-Alfaro *et al*. (1996) suggested that ABA is an important PGR for the accumulation of storage reserves, lipids, proteins, and carbohydrates that have a positive effect in the maturation of somatic embryos.

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Tables

Table (1): Number of somatic embryos and callus fresh weight produced at different concentrations of abscisic acid and polyethylene glycol after 2 months.

Treatments	No. of somatic embryos/ jar	Callus Fresh weight (FW) g/l
0.1 mg/l ABA	6.00	3.387
0.3 mg/l ABA	16.33	2.990
0.5 mg/l ABA	9.33	4.223
1g PEG	14.00	3.887
3g PEG	22.33	4.413
5g PEG	3.66	2.550
LSD 0.05%	3.86	1.700

Table (2): Effect of various concentrations of ABA and PEG on hyperhydricty percentage and number of secondary embryos after 4 weeks.

Treatments	Hyperhydricty Percentage (%)	Germination Percentage (%) (G1)	No. of secondary embryos
0.1 mg/ lABA	63.33	22.20	4.00 B
0.3 mg/l ABA	56.67	11.10	5.33 A
0.5 mg/l ABA	50.00	11.10	6.33 A
1g/l PEG	46.67	66.63	3.33 B

Treatments	Hyperhydricty Percentage (%)	Germination Percentage (%) (G1)	No. of secondary embryos
3g/l PEG	30.00	88.87	5.66 A
5g/l PEG	21.67	55.50	3.66 B
LSD 0.05%			1.229

All treatments were exposed to two hours under laminar flow (Desiccation treatment)

Table (3): Effect of proliferation medium on germination percentage and shoot number after 8 weeks.

Treatments	Germination Percentage (%)(G2)	No. of shoots
0.1 mg/l ABA	100	4.66
0.3 mg/l ABA	88.87	4.66
0.5 mg/l ABA	77.73	3.00
1g/l PEG	100	3.33
3g/l PEG	100	6.33
5g/l PEG	88.87	2.66
LSD 0.05%		1.473

Figures:

Fig.(1): Desiccation of embryos from suspension culture.

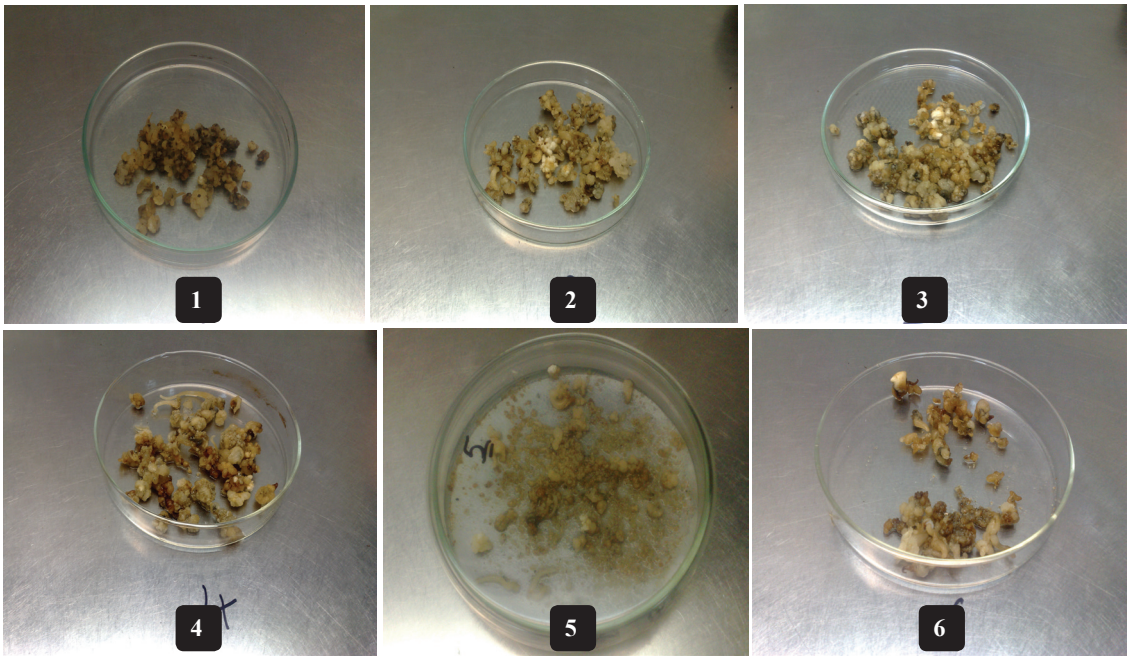

Fig.(2): Production of pro-embryos in suspension culture of date palm using different concentrations of ABA (1) 0.1 mg/l,(2) 0.3 mg/l, (3) 0.5 mg/l and PEG(4) 1 g, (5) 3gm, (6) 5g respectively.

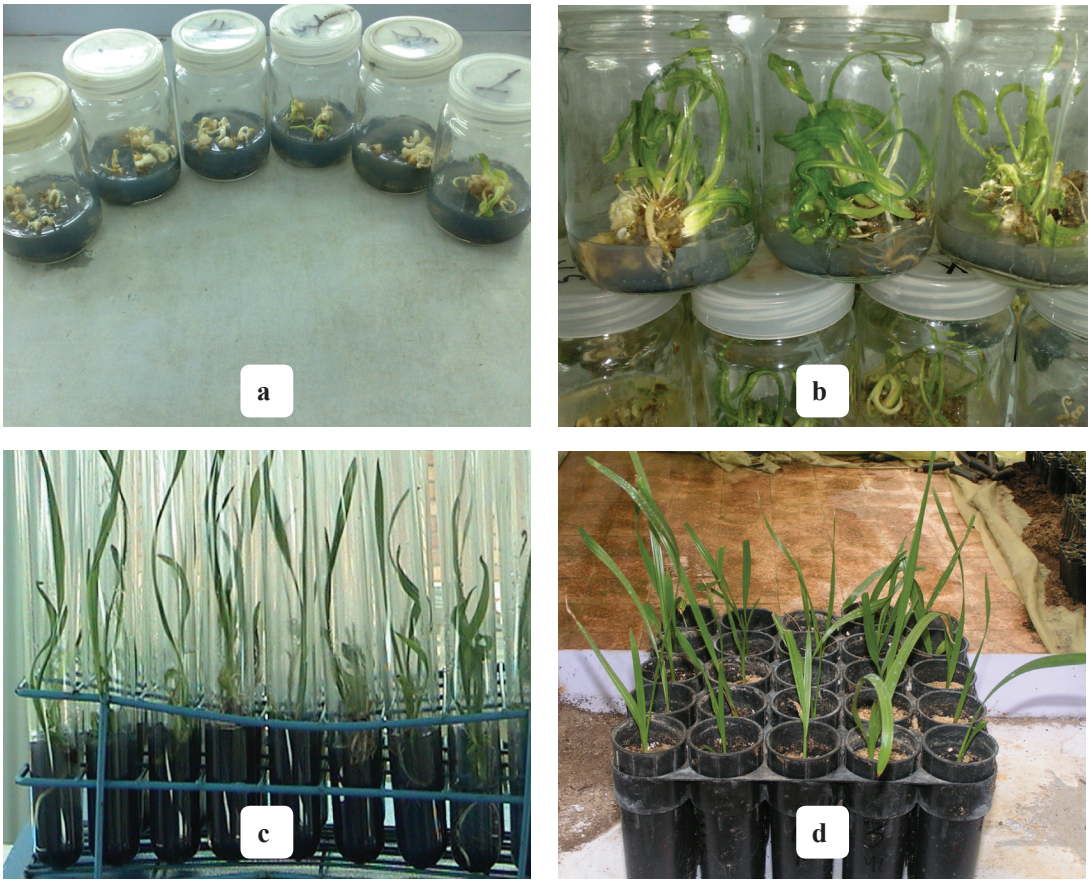

Fig.(3): (a) Different concentration of ABA and PEG on somatic embryos after 4 weeks (b) Shoot proliferation (c) plantlet derived from embryogenic suspension and d) Acclimatization of plantlets.

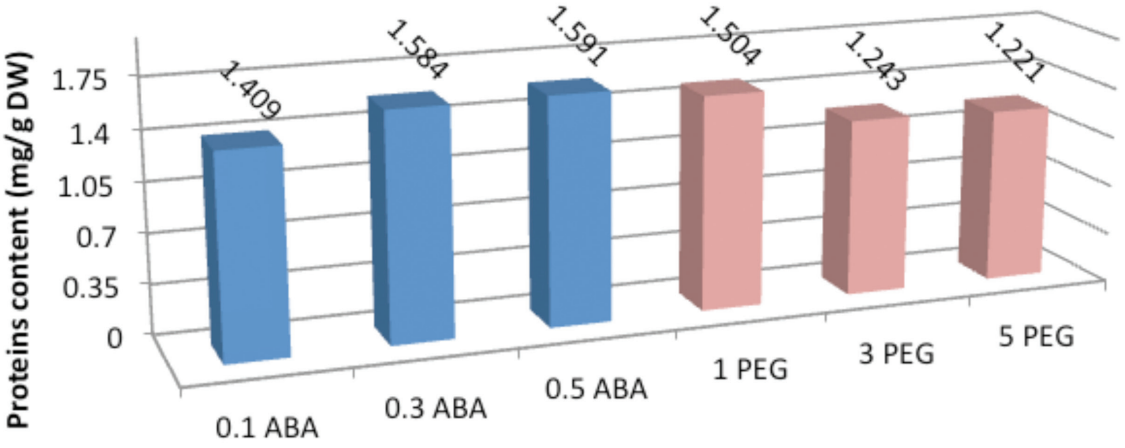

Fig.(4): Protein content of date palm embryogenic callus.

Micropropagation of some Pakistani cultivars of date palm (*Phoenix dactylifera* L.) through inflorescence technique

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ABSTRACT

Pakistan has remarkable position in dates production and export in the world and has very rich date palm varietal structure. Date palm is traditionally propagated by vegetative means because of seeds heterozygosity. Work on propagation of date palm through tissue culture is being done since long back using various explants and techniques. The Date Palm Research Institute (DPRI), Shah Abdul Latif University (SALU), Pakistan has developed an efficient protocol for rapid and large scale in vitro propagation of valuable Pakistani date palm cultivars using Inflorescence explants.

Immature inflorescences of date palm initially washed with low torrent of current tap water and then 30% NaOCl2 solution for surface sterilization, spikelets were cut into the 2-3 cm small pieces and cultured on modified Murashige and Skoog (MS) medium supplemented with 0.1 mg l-1 2, 4-D + 0.1 mg l-1 IAA + 5.0 mg l-1 NAA for initiation and establishment of cultures. Somatic embryos were subjected to multiplication medium involved 0.1 mg l-1 NAA + 0.05 mg l-1BA. Rooting was achieved using quarter strength MS medium containing 0.1 mg l-1 NAA without Activated Charcoal (AC) initiatialy and then with 3 g l-1 AC. Strong rooted plantlets with 2-3 leaves were transferred to pots contained sand and peatmoss mixture (1:1 v/v) with more than 95% success in acclimatization. The acclimatized plants with at least one compound leaf were shifted to the open field conditions. High multiplication efficiency and survival percentage ensure the efficacy of the protocol developed for the production of elite cultivars of date palm of Pakistan.

INTRODUCTION

Pakistan always ranked among top 6 dates producing countries in the world and one of the strongest contenders among the countries claiming place of the date palm origin. Pakistan, particularly Khairpur (Sindh) and Makran (Balochistan) districts have very rich date palm varietal structure. Khairpur district (located in northern Sindh) is famous for its bountiful harvest of dates and often referred to as "city of date palm" and "biodiversity center of dates" having more than 300 varieties (Mahar, 2007; Markhand *et al.,* 2010). Almost 85% of the Sindh dates produced only in Khairpur district (Jatoi *et al.,* 2009).

The Date palm (*Phoenix dactylifera* L.) can be propagated naturally through seeds or offshoots and by Plant tissue culture artificially. Date palm Propagation by seeds always brings Heterozygosity due to its dioecious nature. While using offshoots for commercial propagation facing

limitation of offshoot availability and source of spreading diseases in case if the offshoots taken from infected trees.

A huge number of individual efforts of tissue culture of date palm from both dates producing & non dates producing countries have been reported, particularly from UAE, Egypt, Saudi Arabia, Algeria, Morocco, Tunisia, Pakistan, Iraq, Oman and few from Nigeria, Sudan, Spain, USA etc., but are limited to callus, somatic embryogenesis, multiplication, rooting and only few succeeded to acclimatize plants. At present, a number of public and private sector laboratories concerned with date palm micropropagation on commercial scale such as; Date palm Developments (UK), Al-Rajhi tissue culture laboratory (Saudi Arabia), Al-Ain University date palm tissue culture laboratory (UAE), Marrionet G.F.A (France), Rahan Meristem (Israel), Sapad tissue culture date palm company (Saudi Arabia), Domaine Agricole el bassatine (Morocco), date palm research center (Oman), Green Cost nurseries, Fujairah (UAE), Al-Wathba Marrionet (Iran) producing thousands of tissue cultured plants annually (Zaid *et al.,* 2011; Rajmohan, 2011; Jatoi, 2013).

Usage of the offshoots derived explants in tissue culture of date palm has been practicing since decades. Afterwards, the potential of inflorescence explants have been tested to develop direct (Abul-Soad *et al.,* 2004) and indirect somatic embryos (Drira and Al-Sha'ary, 1993; Abul-Soad *et al.,* 2005) of date palm. Inflorescence explants have many advantages over worldwide frequently used shoot tip explants for date palm micropropagation such as: no or less bacterial contamination, no browning, short production cycle and possibility to produce rare male and elite female cultivars of date palm in case of no offshoots availability (Bhaskaran and Smith, 1992; Abahmane *et al.,* 1999; Feki and Drira, 2007; Zaid *et al.,* 2007; Abul-Soad and Mahdi, 2010; Abul-Soad, 2011; Jatoi, 2013).

The micropropagation of elite local and international marketable varieties in Pakistan is need of the day. The efforts have been made for few decades through dispersed trials in the country to produce date palm plants by tissue culture technology. However, limited success has been achieved and trials weren't fruitful on large scale (Qureshi and Rashid, 1993; Rashid and Qureshi, 1994; Hussain *et al.,* 1995; Qureshi *et al.,* 1997; Hussain *et al.,* 2001; Khan and Bibi, 2012).

The Date Palm Research Institute (DPRI), SALU, Khairpur, Pakistan is working on several aspects of date palm including propagation of local and international varieties through tissue culture using shoot tip and Inflorescence explants. Plant tissue culture laboratory of DPRI has established cultures of many elite varieties of Date Palm in the lab for commercial production range from juvenile to rooting stage and shifted more than 4000 tissue cultured date palm plantlets of various varieties to Glass house (Markhand, 2009; Abul-Soad and Mahdi, 2010; Abul-Soad, 2011). Produced plants were shifted successfully into field conditions for field and fruit evaluation (Jatoi, 2013).

MATERIAL AND METHODS

This work was carried out in the Biotechnology Lab. of Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan in 2007 - 2013. The protocol was done as under:

Plant Source

The immature inflorescences were excised from the mother trees of different date palm cultivars namely Gajar, Kashoo wari and Dedhi (Fig. 1) from Khairpur, Sindh, Pakistan in early spring. The excised inflorescences were kept in clean plastic cover and handled carefully from an open field to the laboratory.

Surface Sterilization & Explant Preparation

The intact spathes were dipped into fungicide solution (2 grams l-1 Topsin M 70) for 30 seconds only without any shaking followed by washing under current tap water for 30-60 seconds only. 30% Sodium Hypochlorite (NaOCl) solution was used as surfactant for 5 minutes and washed three times with sterilized distilled water for 30-60 seconds without shaking.

After sterilization, the outer protective sheath or cover was removed carefully without any damage to the spikes inside. The spikes were cut from their bases and cultured directly if 3-4 cm in length while longer spikelet were cut and divided in to 2-3 cm each of which possessed 2-4 immature florets and laid in such a way that the entire explant is in contact with the surface of nutrient medium.

Cultural Conditions

All cultured explants were incubated in a controlled growth room at 25 ± 2ºC under full darkness and re-cultured about 3-4 weeks on same initiation medium as mentioned in Table 1. Well-responded explants were transferred on to maturation medium for 1-2 re-cultures. Matured and early differentiated explants under darkness were shifted onto differentiation medium under light conditions for 1-2 re-cultures. Subsequently the differentiated cultures were shifted to the multiplication medium to acquire desired number of shoots and then the elongated shoots were detached from multiplication stage and subjected to rooting medium. The individual plantlet with 2-3 leaves and thickened adventitious roots were selected and shifted to the glass house for acclimatization (Fig. 2).

Acclimatization

The acclimatization protocol of date palm was followed as described by Abul-Soad (2011). Plantlets were taken out from test tubes and the roots were gently washed in Luke-warm distilled water to remove any residual gel or medium. Before planting, plantlets were immersed in 0.5% (w/v) fungicide solution for 5 minutes. The plants were placed into 250 mm plastic pots containing soil mixture 1:1 of wash sand: peat moss (v/v) with little amount of Perlite. Plants kept under natural day light and high relative humidity (90-95%) using a cover of white polyethylene sheet for one week and removed gradually to develop the plants under greenhouse conditions. The plants were watered once a week and sprayed with the fungicide if needed.

RESULTS AND DISCUSSION

The current study was conducted to achieve successful large scale micropropagation protocol of date palm using inflorescence explants. No browning and bacterial contamination observed during initiation phase and all spike explants responded well to the starting nutrient medium. Shining globular creamy structures formation was obtained within 2 months through 1-2 re-cultures. Maturation of initial structures occurred within 2-3 months through 2-3 re-cultures. After the differentiation process, three types of cultures were obtained e.g. embryogenic callus, somatic embryos and green shoots. Somatic embryos can be generally divided into two categories. First category is the individual somatic embryos and second is a cluster of embryos (multiple embryos). The growth behavior of the individual embryo is to grow vertically to produce more leaves and roots while the multiple embryos is usually proliferating to additional shoots and somatic embryos which suits the multiplication stage.

In the first subculture of multiplication stage, 110, 24 & 70 jars were having embryogenic callus in cvs. Kashoo wari, Gajar & Dedhi, respectively. While, with 38 jars having multiple embryos cv. Kashoo wari appeared the only variety produced embryos. However, 28, 2 & 17 jars of cvs. Kashoo wari, Gajar & Dedhi were appeared with shoots respectively (Table 2). All of these cultured were transferred onto the proliferation medium (Table 1). The embryogenic callus exposed high morphogenetic potentiality to differentiate to intact somatic embryos. During this process very little callus formation was occurred till subculture 7 of multiplication stage where the callus jars decreased to 5, 0 & 6 jars in cvs. Kashoo wari, Gajar & Dedhi respectively. While, with 111 and 408 jars of embryo and shoot cv. Kashoo wari produced prominent number of cultures as compared to cv. Gajar (44 embryo and 119 shoot jars) and cv. Dedhi (31 embryo and 86 shoots jars). During multiplication stage some shoots were growing up and reached to an appropriate height for rooting stage and subsequently subjected to the rooting medium (Table 1). It was observed that removing the initial roots completely or trimming to 1-2 mm enhanced thicker-white adventitious root formation. Leaving the primary roots without trimming during rooting stage inhibited the adventitious roots formation which is important for the further growth in the acclimatization stage (Abul-Soad and Jatoi, 2014).

Finally, all callus and embryos differentiated into shoots and rooted plantlets on rooting medium. Where number of shoots and plantlet were reached at 419 and 773 in cv. Kashoo wari, 299 and 295 in cv. Gajar, and 50 and 257 in cv. Dedhi, respectively.

The shoot jars increased from 28, 2 & 17 in subculture 1, then 408, 119 & 86 in subculture 7, and 419, 299 & 50 in subculture 12. Each jar maintained 20-30 shoots, 5 of them at least in the size of rooting stage while 1325 plantlets were in rooting stage in sub culture 12. It is quite important to mention that no any study has been conducted on these date palm cultivars before and offshoots were only the source of traditional propagation method in the region.

Rooting quality of the ex vitro plantlets of date palm was the vital factor increased the survival percentage in the greenhouse. Most of the reports indicated low survival percentage 25-35% during acclimatization stage rather than it used to be a big obstacle in the whole micropropagation protocol (Abul-Soad *et al.,* 1999; Hegazy *et al.,* 2006; Taha *et al.,* 2007). But in current study and based on the utilization of high sugar concentration, AC after adventitious roots formation and proper handling for the plant material, the survival percentage reached more than 95%. The used soil bed was a simple mixture of washed sand and peat-moss (1:1 ratio) with little amount of perlite. The acclimatized plants with at least one compound leaf were shifted to the field conditions (Fig. 3-5). High multiplication efficiency and survival percentage ensure the efficacy of the protocol developed for the production of elite cultivars of date palm of Pakistan.

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Table 1: Nutrient media composition for Inflorescence protocol and its sequence (Abul-Soad and Mahdi, 2010)

Medium	Composition (mg l^{-1})			
	Salts	Additives	Auxins	Cytokinins
Initiation	Macro of B5[z]+ Micro of MS[y]	30000 Suc.[x] + 2200 Agar + 1400 Gel + Vit.[w] of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Ad.[v]	0.1 2,4-D + 0.1 IAA + 5.0 NAA	---
Maturation	Macro of B5+ Micro of MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Ad. + 1500.0 AC[u]	5.0 2,4-D	1.0 2iP
Differentiation	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA	0.1 Kinetin
Multiplication	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA	0.05 BA
Rooting	*¼ MS*	50000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 0.1 Ca-panthothianate + with & without 3000.0 AC	0.1 NAA	---

[z]B5: Gamborg et al. (1968) nutrient medium. [y]MS: Murashige and Skoog Medium (1962). [x]Suc.: Sucrose. [v]Vit.: Vitamins. [w]AC: Activated Charcoal. [u]Ad.: Adenine sulfate.

Table 2: production capacity of three different cultivars of date palm from a single inflorescence after 1, 7, 12 subcultures during the multiplication and rooting stages.

Variety	Sub 1*				Sub 7				Sub 12		
	Callus	Embryo	Shoot	Total	Callus	Embryo	Shoot	Total	Shoot	Plantlet	Total
Kashoo wari	110	38	28	176	5	111	408	524	419	773	1716
Gajar	24	---	2	26	---	44	119	163	299	295	757
Dedhi	70	---	17	87	6	31	86	123	50	257	430
General Total	204	38	47	289	11	186	613	810	768	1325	2903

*Sub 1 considered during multiplication stage.
Each culture vessel (350 ml jar or 250 × 25 mm long tube) contained 1 gram of callus, or 10 embryos or shoots in average. Each long tube contained 1 intact plantlet with shoot-root system.

Gajar | Kashoo wari | Dedhi

Fig. 1 The fruit of studied cultivars used for micropropagation

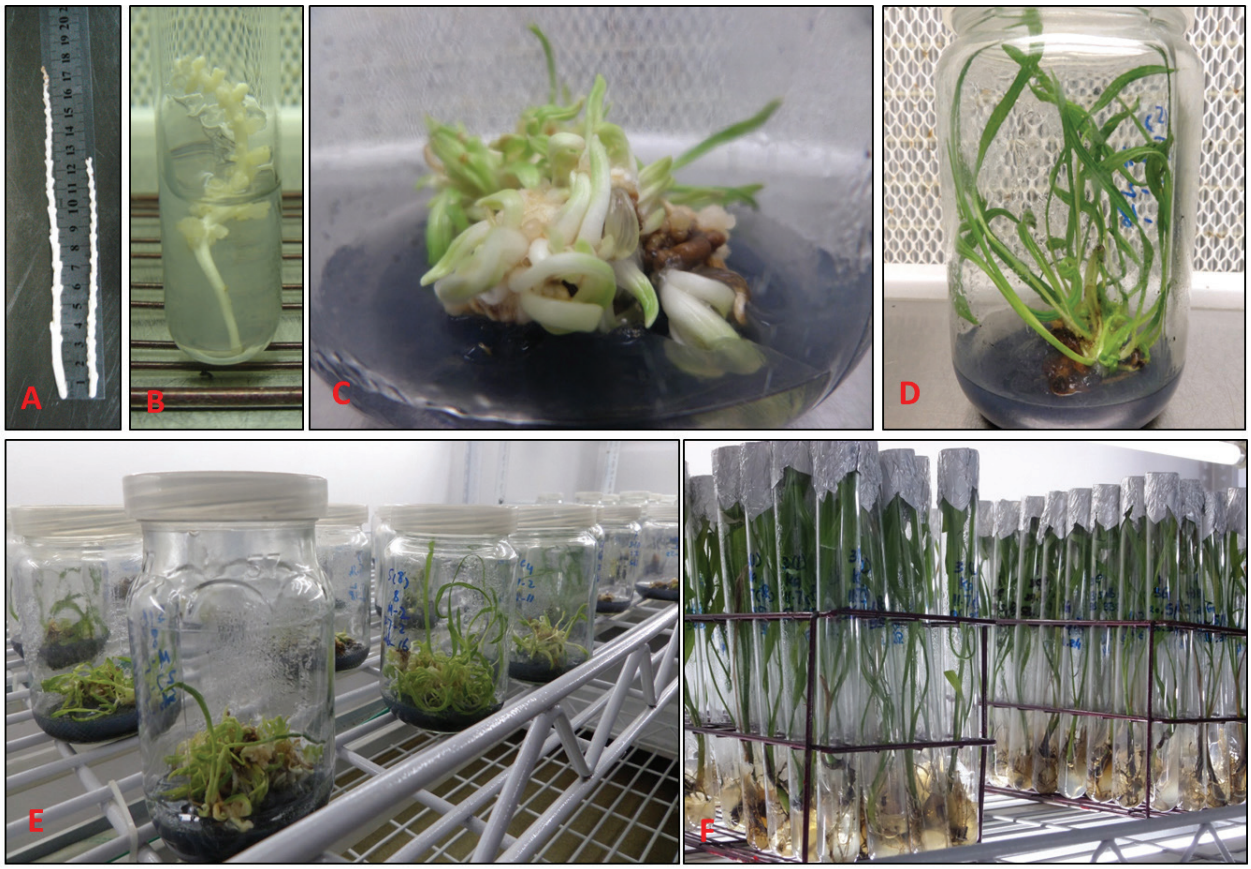

Fig. 2. Different growth stages of date palm micropropagation using Inflorescence explants in DPRI. A. Inflorescence Spikelets B. Initiation stage C. shoots cluster with somatic embryos D. shoots elongation E. Multiplication stage E. Rooting stage.

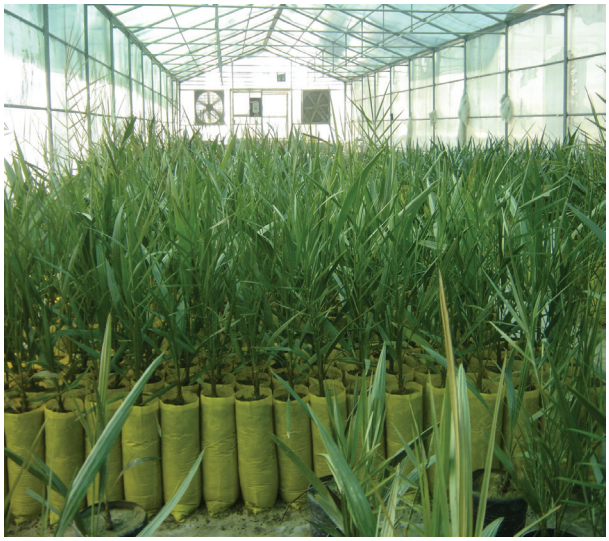

Fig. 3. Date palm plantlets acclimatization process in DPRI Glass house.

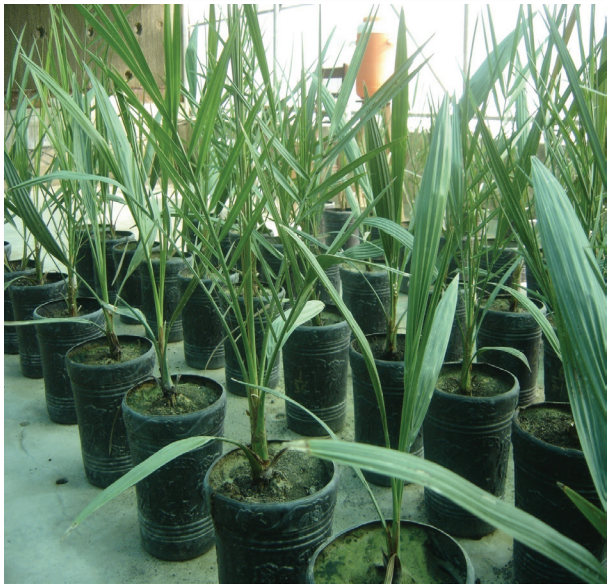

Fig. 4. Tissue cultured date palm with compound leaves ready to be shifted to field conditions.

Fig. 5. Tissue culture derived date palm plants in field conditions, DPRI, SALU, Khairpur, Pakistan.

Honeybee venom in control of the microbial contamination of date palm tissue culture stages

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ABSTRACT

***Penicillium* sp., *Aspergillus* niger, Bacillus sp. and Pseudomonas sp. Were isolated from *Phoenix dactylifera* cv. Haiany culturing in vitro in multiplication and rooting stages.**

Six dilutions of honeybee venom (1/10, 1/100, 1/1000, 1/10 000, 1/ 100 000, 1/1000 000 mg L-1) were used to control the Fungal and Bacterial contamination during the date palm tissue culture stages (Multiplication and Rooting stage) without affecting the explants response and development as happened after adding certain antibiotics to the medium. Contaminated explants were cultured on venom containing media for one subculture (4 weeks). After this period explants were subculture onto medium-free venom to study its residual effect on explants contamination.

The results recorded that the first dilution 1/10 has a great effect on controlling the contamination but it reduced the explants response to the media. The dilutions 1/100, 1/1000, 1/10 000 were most effective for controlling the contamination compared with other treatments under investigation without affecting the explants response and development. While the last two dilutions had no effect on decreasing the contamination. When the explants were subculture on to free venom-medium or supplemented with high dilution as 1/1000 and 1/10 000 contamination was recognized in few gars.

Key words: honeybee venom, date palm, tissue culture, contamination

INTRODUCTION

Date palm (*Phoenix dectilefera*) is being propagated traditionally by using seeds or off shoots. Unable to satisfy the extension in new land of the desert as seeds give approximately 50% males and the females are not genetically similar to the mother and off shoot produced in few numbers through life time and along with losing numbers during rearing and transporting, making the tissue culture technique most promising for mass production of the date palm with high quantity and supreme quality especially for rear and expensive species. (veramendi and Navarro,1996) .

Preparing the plant to use in tissue culture needs certain presages precaution since it will carry over organisms from the field or being contaminated accidentally by the staff who undertakes the propagation.

Tissue culture medium is a suitable hostile to various forms of bacteria and fungi, because of high sugar content in it. Under these circumstances certain species of bacteria and fungi are being favored by the optimized growth condition and challenging the value of tissue culture technology. (Barnett and Hunter, 1986)

The use of Clorox and other chlorine containing substance, through effective in controlling contamination have been complained about producing maceration of plant tissues of certain varieties along with the health hazards to the technical staff and environment safety. Therefore, the bee venom as natural antibacterial agent medically approved for human use was searched for in this study to replace the antibiotics in tissue culture

technology to overcome contamination and minimize the hazardous effect of chlorine at the recommended dose of concentration. (Abd-El Kareim *et al.,* 2006)

Bee venom are more friendly than Clorox (sodium hypochlorite Na HOCL) 4.5-5% with no residual effects. And less coasting than it. Using bee venom added 30ps to the total coast of the medium while using Clorox added 60-70ps to medium per litter.

Honeybee venom had an effect Gram-Negative and Gram Positive bacterial species without affecting the plants as happen when using the being recorded with antibiotics (Boman *et al.,* 1989: El-Shaarawy, 2008). Also It had an effect against fungi (Surendra *et al.,* 2011: A-Reum Yu, 2012), which au less sensitive to antibiotics.

And with using of antibiotic widely in controlling of plant tissue culture contamination for about 50 years made resistance to antibiotics beside it affects on the plant health (Katznelson and Sutton, 1951), (Abd-El KareimA.H.*et al.,*2006)

MATERIALS AND METHODS

The present study was carried out during 2012 – 2013 at Central Laboratory of date palm research and development, Agriculture Research Center, Giza, Egypt, and the bee venom was collected from private bee yard from El- Qalubia.

1. Bee venom preparing:

The honeybee venom was collected from bee hive using electric shook device, and stored as powder ready to use. The bee venom was diluted in distilled water to the different concentrations. (El-Shaarawy, 2008)

2. The contamination:

Explants contaminated with fungi and bacteria were collected from two different stages (Multiplication and Rooting) under investigation and then planted into ready potato-dextrose agar (PDA) plates and incubated at 25◦C for five days. The isolated fungi were identified using the description of (Barnett and Hunter, 1986). Identification of the isolated fungi was confirmed at the Mycol. and Dies. Survey Res. Dept. Agric. Res. Center Giza. The isolated bacteria was identified using the description of (Breed *et al.,* 1974) and confirmed at the Bacteria Diseases Res. Dept. Agric. Res. Center Giza.

3. Preparing of the media:

Four kinds of media were prepared two for each stage:

Multiplication stage: a) MS + 30 gm L-1 sucrose + 3 mg L-1 2ip + 3 gm active charcoal b) MS + 30 gm L-1 sucrose + 3 mg L-1 2ip + 3 gm active charcoal + venom.

Rooting stage: a) MS + 1 mg L-1 NAA + 30 gm L-1 sucrose + 3 gm active charcoal b) MS + 1 mg L-1 NAA + 30 gm L-1 sucrose + 3 gm active charcoal + venom.

These media were autoclaved for 20 min. at 121°C (1.2 kg cm-1) in flasks. Let the flasks to cool down to 40°C inside the laminar flow then added the different concentrations of bee venom using syringe filter with 0.02 micron then the flasks were shaken well for perfect distribution of bee venom dilutions 1/10, 1/100, 1/1 000, 1/10 000, 1/100 000, 1/1 000 000 mg L-1 .

The media were distributed in jars 40 ml / jar then incubated for 4 days before using (planting).

4. Choosing the plants:

Contaminated explants with bacteria were collected from the two different stages under investigation. There were 20 jars for every concentration of venom from 1/10 to 1/1 000 000in every stage of the palm tissue culture with 20 jars as control replanted on the same medium (with total of 240 jars). All chosen jars had a medium bacterial contamination with suitable healthy plants.

The explants were first cleaned up in distal sterilized water then planted on the media. Explants were incubated in growth room at 26 ± 2°C in 16 hr. illumination of 2000 lux (white flour cent lamps). Sub culturing the explants were done every 3 weeks 5 jars on free venom medium and the rest on the tested medium to determine the effect of bee venom on the contamination and the response and the growth of the explants.

Data were taken as follows: Survival %, Contamination by bacteria.

RESULTS AND DESCUTION

The effect of bee venom on the contamination:

I: The multiplication stage:

Data present in table (1) showed that effect of bee venom difference concentrations against the bacterial and fungal contamination in the multiplication stage. The first three dilutions 1/10, 1/100 and 1/1000 gave a good effect on the contamination on the tissue culture jars as there were no growths of the bacteria or fungi in the jars even when transplanting some of the explants on free venom medium (control). 1/10 000 concentration was effective as he present in the medium in the three subculture but when the explants planted on free medium some jars got contaminated in the 2nd and 3rd sub. The last two concentrations 1/100 000 and 1/1000 000 were not effective on the bacterial or fungal contamination as it appear badly on them.(Fig 1 and 2)

II: The rooting stage:

Data in table (2) shows the effect of bee venom on with the rooting stage. In the four concentrations 1/10, 1/100, 1/1000 and 1/10 000 there were no growth of bacteria in the tubs and after replanting them on the control medium too. But with the last two concentrations 1/100 000 and 1/1 000 000 the venom was not effective on the bacteria and there were many contaminated tubs. But it can be observed that the contaminations are less in rooting stage than multiplication stage. (Fig 3 and 4)

The privies results of bee venom are in agreement with many authors (Boman *et al.,* 1989; El-Gizawy 2006; El-Shaarawy 2008). They all founded that some of the honeybee venom got an effect on the growth of bacteria. And with Mulfinger, 1990 and Kaviani *et al.,* 1995 found that big dilutions of bee venom become not effective on growth some kind of bacteria.

But with the observation the plants in dilution 1/10 were affected by the bee venom and there growth was less than normal in the other dilutions or in control.

From the previous results, it can demonstrated that, the best bee venom concentration to use in date palm tissue culture are 1/100 and 1/1000 concentrations as it control the bacterial or fungal contamination and not does affect the plant vigor.

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Tables

Table (1): The effect of bee venom on contamination of multiplication stage contamination jars of date palm tissue culture.

Venom dilutions	Contaminated jars	1st sub		2nd sub*		3rd sub	
		Free◊	Cont.□	Free	Cont.	Free	Cont.
Control	20	-	20	-	-	-	-
1/10	20	20	-	15	-	10	-
Control				5	-	10	-
1/100	20	20	-	15	-	10	-
Control				5	-	10	-
1/1000	20	20	-	15	-	10	-
Control				5	-	10	-
1/10000	20	20	-	14	1	8	2
Control				3	2	5	2
1/100000	20	15	5	6	4	4	2
Control				-	5	-	-
1/1000000	20	10	10	-	5	-	-
Control				-	5	-	-

◊Free = no. of free contamination jars □ Cont. = no. contaminated jars
*Sub: re culture after 45 days on the same midia

Table (2): The effect of bee venom dilutions on contamination of rooting stage of date palm tissue culture.

Venom dilutions	Contaminated jars	1st sub		2nd sub		3rd sub	
		Free◊	Cont.□	Free	Cont.	Free	Cont.
Control	20	-	20	-	-	-	-
1/10	20	20	-	15	-	10	-
Control				5	-	10	-
1/100	20	20	-	15	-	10	-
Control				5	-	10	-
1/1000	20	20	-	15	-	10	-
Control				5	-	10	-
1/10000	20	20	-	14	1	8	2
Control				3	2	2	2
1/100000	20	15	5	7	3	2	2
Control				1	4	1	2
1/1000000	20	12	8	5	2	1	2
Control				1	4	-	2

◊ Free = no. of free contamination jars □ Cont. = no. contaminated jars

Figures

Fig. 1. Contaminated date palm plants before replanting on bee venom medium (multiplication stage).
Fig. 2. Contaminated date palm plants after replanting on bee venom medium (multiplication stage).

Fig. 3. Contaminated date palm plant before replanting on bee venom medium (rooting stage)

Fig. 4. Contaminated date palm plant after replanting on bee venom medium (rooting stage).

Promising protocol for *in vitro* direct organogenesis of date palm cv. Ekhlass

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ABSTRACT

Over the last forty years, serious attempts and efforts were made to develop direct organogenesis method through axillary buds growth and development pathway. Direct organogenesis has been considered the protocol for distinguishing high genetic stability and true-to-type date palm vitroplants. To achieve this goal, specific poking and several incisions that were made mechanically at specific time on the shoot tips apices explant of date palm (*Phoenix dactylifera* L.) cv. Ekhlass eliminated apical dominance and promoted axillary buds growth.

The shoot tips were cultured initially for 2 or 4 weeks on a medium-free hormone. While, axillary buds proliferated on the explant re-cultured monthly, for three month, on modified MS (Murashige and Skoog 1962) medium (MMS) supplemented with BA (1.0 mg/L), 2iP (1.0 mg/L), Kin (1.0 mg/L), NOA (0.5 mg/L), solidified with gelrite (2.0 g/L), and incubated at 25±1°C under dark conditions. Propagules cultured on the previously described medium, omitting BA plus NOA and supplementing with 2iP, Kin and IAA at (0.2 mg/L), putrescine (50 mg/L) and adenine hemi-sulphate (160 mg/L) recorded significantly the highest axillary buds multiplication rate, and growth value, after incubation under 16-h photoperiod using fluorescent tubes with a light intensity of 1500 Lux for 8 weeks. Separated shoots, cultured on basal MS medium in addition to NAA (O.2 mg/L), spermidine (100 mg/L), sucrose (30 g/L) and solidified with phyto- agar (6.0 g/L), recorded the highest significant roots number, and roots length after 4 weeks of incubation. Healthy rooted plantlets, hardened by receiving pre-acclimatization treatment through transferring from agar rooting medium to 1/2 MS salts strength liquid medium and raised light intensity to 8000 Lux (natural light) for 4 weeks, had the highest survival percentage values in a mixture containing compost and perlite (1:1, v/v) after 3 months in acclimatization.

Keywords: *Phoenix dactylifera* L., tissue culture, micropropagation, axillary buds,polyamine (PAs).

INTRODUCTION

Expansion of date palm agriculture is faced with challenges stemming from propagation and genetic improvement limitations. The heterozygous nature of this dioecious species hampers the use of seeds which produce off type seedlings. On the other hand, the limited availability of offshoots and the difficulties of establishing propogules from offshoots render this traditional propagation method inadequate, particularly for large-scale propagation. Based on recent advances in plant tissue culture, micropropagation technique has been developed for the rapid mass propagation of date palm. Some limitations associated with genetic improvement have been circumvented by taking advantage of tissue culture applications and molecular methodologies. Somaclonal variation occurring is quite common in date palm micropropagated plants produced through indirect embryogenesis pathway, but it can be controlled by *in vitro* direct pathway practices (Jain *et al.,* 2011).

It is essential to provide the candidate famers, not only well adapted cultivars, but also they must be true-to-type tissue culture-derived date palms that do not present any abnormalities. This true-to-typeness is guaranteed when the tissue culture palms have been produced by strict organogenesis (without any callus formation). The most important abnormalities are infertility of the female flowers and dwarfism. For the cv. Barhee, the batches produced by some laboratories were 100% abnormal (Al-Wasel 2001). A mixed embryogenesis/ organogenesis method could also eliminate the risk of abnormalities development (Ferry *et al.,* 2000).

One of the main technological factors limiting the use of this technique is the production of abnormal plants, when plantlets are obtained by somatic embryogenesis. At the moment, the shoots that are supplied with a true-to-typeness guarantee are produced by organogenesis. However, this technique does not offer the same propagation speed. It is a high labor-consuming process and consequently a costly one. Furthermore, very few laboratories control organogenesis at an industrial scale. This slow development associated with a participative-approach strategy is usually better adapted to the small farmer capacity and, quite preferable to the uncontrolled, unreasonable and unsustainable date palm plantations growth that has been adopted in some countries over the last 30 years (Ferry, 2011).

Although research in date palm biotechnology is relatively limited, specially through direct pathways. It is evident that direct organogenesis is a promising technique and will reflect significant influence in date palm true- to- type plant production (Ibrahim and Hegazy, 1999 and 2001).

The aim of this work was to study the availability of micropropagate the high quality date palm cv. ‘’Ekhlass’’ through a complete promising protocol for axillary buds proliferation pathway.

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Dept. of the Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City Sadat City, during the period 2011- 2013.

Explant materials used in this study were obtained from 4 years- old offshoots of female date palm cv. “ Ekhlass” have high quality fruits grown at, Mr. Nabil Abdrabboh model farm, Al-Katta Desert, Gizza governorate, Egypt. By using a hatchet and a sharp knife, the leaves and fiber sheath were removed acropetally. Ethyl alcohol (70%) was sprayed over cutter and plant material during dissection process. When the final size were about 5 cm in width, and 10.0 cm in length, the selected plant materials were excised and inverted upside down and sprayed with alcohol. Then, soaked in sterilized antioxidant solution (150 mg/L each of citric acid and ascorbic acid). Then kept in the refrigerator at (5.0 C°) until the surface sterilization procedure is performed. Surface sterilization were implemented twice by soaked in 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 rinsed with sterile distilled water. Another post- treatment used was 0.1 % Mercuric chloride solution containing two drops of tween -20 per 100 ml for 10 minutes. Explants were then rinsed three times with sterile distilled water and finally soaked in sterilized anti-oxidant solution till final dissecting. Additional leaves were removed when the cluster of very little leaves of the apex is reached and obtaining the shoot tip with a base of meristele tissue, then cultured shoot tip with a good contact on the surface of the medium.

Nutrient Media

Shoot tips explant isolated from offshoots were cultured on modified MS basal medium (Murashige and Skooge, 1962) supplemented with [asparagen (100 mg/L), glutamine (200 mg/L), bioten (0.5 mg/L), adenine himi-sulphate (80 mg/L), thiamine-HCl (10 mg/L), Ca-pantothenate (10 mg/L), a ascorbic acid (75 mg/L), citric acid (75 mg/L), NaH2PO4. 2H2O (170 mg/L), activated charcoal (1.5 g/L) and raised sucrose up to (40 g/L)] was used. The pH of the solid media was adjusted to 5.6 and 5.2 for liquid medium with 0.1 M KOH or 0.1 M HCl prior to gelling agent addition. Media were dispensed either in jars (150 and 350 ml) in aliquots of 30 and 50 ml/jar respectively, covered with polypropylene closure. or into a glass tubes (25 x 2.5 cm; Borosil) capped with Bellco plastic caps containing 15 ml and autoclaved at 121°C and 1.2 Kg/cm^2 for 20 min.

1- Effect of right timing for induce shoot tip poking and incisions.

Shoot tips explant were cultured individually on modified MS medium (MMS) free-hormone solidified with gelrite (2 g/L). Poking and several incisions were done mechanically to shoot tips; directly with culture or after 2 or 4 weeks from culturing. Nine jars 150 ml (replicates) were used for each treatment. Cultures were incubated in total darkness at 25±1°C. Data of fresh weight (g) and degree of browning were recorded after 6 weeks for all treatments. Crude Protein (%),Total amino acids (%), Total soluble carbohydrates (%), Total soluble phenol (g / g protein) and PAL activity (nkat / g protein) were analyzed and recorded.

2- Effect of some growth regulators apical dominance break down and axillary buds growth:

Shoot tips resulted from the best previous treatment were subjected individually on MMS medium supplemented with cytokinins [BA (6-benzyladenine), Kin (6-Furfurylaminopurine) and 2iP (6-(ŷ,ŷ-dimethylallyl amino purine)]. Auxin [NOA (Naphthoxy acetic acid)] 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 etch treatment. Cultures were incubated at the same conditions previously mentioned and recultured 3 times to the same medium 4 weeks intervals. Data of fresh wt. (g) and axillary bud proliferation %, total soluble phenols and phenylalanine ammonialyase (PAL) activity were recorded.

3- Effect of putrescine and adenine hemi-sulphate on axillary buds multiplication rate and growth value.

Grown shoot tips resulted from the best previous treatment were subjected individually on MMS medium omitted BA and NOA supplemented with 2iP, kin and IAA at the concentration (0.2 mg/L), putrescine (50, 100 and 150 mg/L), adenine hemi-sulphate (80 and 160 mg/L). Nine jars (350 ml) were used as replicates for etch treatment. Cultures were incubated under 16-h photoperiod using fluorescent tubes with a light intensity of 1500 Lux for 8 weeks and recultured monthely. Data of axillary bud formation, axillary buds multiplication rate, fresh weight(g) and growth value were recorded.

4- Effects of spermidine concentrations on rooting stage

Individually proliferated shoots were cultured on basal MS medium supplemented with sucrose (30 g/L) in addition to NAA (naphthalene acetic acid) at the concentration of 0.2 mg/L and different levels of spermidine (0, 50, 100 and 150 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 4 weeks, data of root formation %, no. of roots and root length (cm), total soluble phenols and phenylalanine ammonialyase (PAL) activity were recorded.

5- Acclimatization of plantlets using different growing mixture types:

Plantlets produced from rooting medium after 4 weeks were rinsed under tap water and the roots system immersed in fungicide (Tashgarin) solution (0.5 %, v/v) for 5 min. Then, planted in plastic pots (5 ×18 cm) filled with different growing mixture types as follows: compost and bark chips (1:1, v/v), compost and perlite (1:1, v/v), compost and coconut shell and finally, compost and rice shell (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 (70 %) and 16 h photoperiod with a light intensity of 1500 lux. Acclimatization was achieved through gradually removing the plastic sheet each day till it totally removed after 30 days. Plants were transferred to 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 (Kristalon, 1.0 g/L) at a ratio of 20: 20: 20. After 3 months saplings survival %, no. of leaves/plant and leaf area (cm2) were recorded.

6- Acclimatization with using specific pre-treatments for hardening

Rooted plantlets produced in 4 weeks in the best previous rooting agar medium contained NAA 0.2 mg/L and spermidine 100 mg/L were subjected to pre-acclimatization process through hardening by reculture in 1/2 MS salt strength liquid medium for 4 weeks and received natural light intensity (8000 Lux) in the greenhouse. Acclimatization was achieved typically as previously mentioned on the best results of the growing mixture (compost and perlite). After 3 months saplings survival %, no. of leaves/plant and leaf area (cm2) were recorded.

Degree of browning: It was determined according to the rate of scalling by Pottino (1981), which included, no browning (1), average browning (2) and high browning (3).

Growth value: Explants growth value was determined according to the equation of Ziv (1992).

Ziv (1992). $GV = \frac{Fw_F - Fw_i}{Fw_i}$ Where's

GV = Growth value. FwF = Final fresh wt. Fwi = Initial fresh wt.

Chemical analysis:

Crude protein (usual micro kjeldahl methods) were determined according to the methods described by (A.O.A.C. 1990). Total amino acids were determined according to

the method of Rosein (1957). Total soluble carbohydrates was determined in the ethanolic extract using the phenol-sulfuric acid method according to Dubois *et al.,* (1956).

The colorimetric method of Folin-Denis as described by Swain and Hillis (1959) was employed for the determination of total soluble phenols in ethanolic extracts of leaf samples. Total tannins were determined calorimetrically as described by (A. O. A. C. 1995). Extraction and assay of phenylalanine ammonialyase (PAL) were done according to Lamb *et al.,* (1979).

Statistical analysis:

Data of all studied experiments were statistically analyzed by one factorial randomized complete design using the SAS (1988) package. The Least Significant Difference among levels of each treatment were compared using L.S.D. test at 5%, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Over the last forty years, serious attempts and efforts were made to develop direct organogenesis method through axillary buds growth and development pathway. Direct organogenesis has been considered the protocol for distinguishing high genetic stability and true-to-type date palm vitroplants. As a result of reviewing a large numbers of date palm micropropagation studies, which demonstrated the *in vitro* pathways. It is predicted this report may consider as the first practical complete protocol on direct axillary buds proliferation.

1- Effect of right timing for induce shoot tip poking and incisions.

It could be noticed different behaviors from shoot tips grown after culture depending on timing to induce poking and incisions to the shoot tips, it was very critical for the obtained responses.

Data presented in Table (1) and Fig. (1- a) indicated that, inducing poking and incisions to the shoot tips cultured resulted in different response and the timing was very critical factor for eliminate oxidative browning which play an important role in shoot tips growth and subsequently axillary buds multiplication. Inducing poking to the shoot tips 2 weeks after culture resulted the lowest significant degree of browning and the highest significant value of total Amino Acids (5.92 %), crude Protein (37.31%), total Soluble carbohydrates (19.41 %), total soluble phenol (4.95 mg / g f. wt.) and PAL activity (112.12 nkat /g protein) as compared with the other studied timing 0 or 4 weeks. On contradictory, inducing poking and incisions to the shoot tips after 4 weeks from culturing induced severe oxidative browning and subsequently significant negative response and the lowest significant value of total Amino Acids (2.87 %), crude Protein (17.90 %), total Soluble carbohydrates (13.11 %), total soluble phenol (0.78 mg / g f. wt.) and PAL activity (57.24 nkat /g protein) as compared with the other studied timing 0 or 2 weeks. Results strongly indicated that oxidative browning may the critical point which could affect negatively all the parameters under study. Hegazy, 2003 reported that in date palm tissue culture, injury through separation of explant tissues is accompanied by release of discoloring substances in the medium which may have profound physiological disorders on the cultured tissues and accompanied by an increase in growth value and higher concentration of total soluble phenols. The inhibitory action of phenol may results from its oxidation to quinones by polyphenol oxidase and peroxidase and subsequent binding with proteins, such process may lead to the loss of various enzyme activities (Hu and Wang, 1983). In his regard, Zaid (1984) who found that addition of activated charcoal and PVP in the culture media and subculturing on fresh medium for short periods of incubation prevented explant browning.

2- Effect of some growth regulators on apical dominance break down and axillary buds growth:

Shoot tips received poking and several incisions 2 weeks after culturing obtained positive response Table (2) and Fig (1-b & c) when reculture three times under dark conditions on MMS in addition to NOA (0.5 mg/L) in combination with equal level of BA (1.0 mg/L), kin (1.0 mg/L) and 2iP (1.0 mg/L) were recorded the highest significant values of fresh wt. 17.13 (g) and higher significant percentage values of axillary bud growth (33.33%), this was accompanied by the highest significant values of total soluble phenols (4.77 mg/g f.wt) and phenylalanine ammonialyase (PAL) activity (99.47 nkat /g protein) as compared with the other studied individual growth regulators treatments. Total soluble phenols and PAL activity contribute to the formation of protective materials, i.e. lignin, suberin and flavonoids, which would in somehow affect the speed of plant development. In this concern, 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 is indicates giving true- to-type plantlets. 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). culturing explant *in vitro* 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 (Ziv, 1991). Moreover, Zaid and Tisserat (1983) reported that, addition of growth regulators

to nutrient medium was not necessary to stimulate shoot proliferation, better shoot tip development occurred on 10 and 100 mg/L NAA. On the other hand, Tisserat (1984) reported that addition of cytokinin at any level to date palm tissue culture media did not enhance shoot differentiation.

3- Effect of putrescine and adenine hemi-sulphate on axillary buds multiplication rate and growth value.

Growing axillary buds upon transfer to light conditions for 8 weeks on previous medium omitted BA and NOA while supplemented with IAA, 2iP and Kin at equal concentrations (0.2 mg/L), in addition to putrescine (50 mg/L) and adenine hemi-sulphate (160 mg/L) resulted in significant axillary bud multiplication rate (2.11 buds /shoot tip) and the highest fresh weight (25.2 g) and growth value (727) as compared with the control and the other studied concentrations treatments (Table 3 and Fig. 1- d & e). It could be noticed that adenine sulphate at low level in combinations with all putrescine concentrations had negative response to induce axillary bud multiplication. Clusters of shoots Fig. (1- f) were transferred to the same medium for 4 weeks elongation. Similar results were published by Hegazy (2008) 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 PAL activity. Hegazy and Abo shamaa, (2010) on achieved direct date palm embryos cv. Medghool on modified MS medium contained putrescine (100 mg/L) . in this regard, 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. In contradictory, Handa and Mattoo (2010) on tomato they found that the diamine putrescine generally contrast those with polyamines spermidine and spermine emphasizing that individual biogenic amines should be considered to have defined action in plant biology and that they differentially affect growth and development. In this regard, Al Kaabi *et al.,* 2007 reported that organogenesis and somatic embryogenesis are the two techniques currently used in various laboratories in the world for in vitro mass propagation of date palm. Organogenesis in date palm has a low efficiency due to the low number of explants that respond in vitro, the long time required for the initiation phase, the low multiplication rate and the strong influence of the variety (Beauchesne *et al.,* 1986).

4- Effects of spermidine concentrations on root growth characters and chemical analysis

Resulted data in Table (4) and Fig. (1- g) showed that, shoots cultured on basal MS medium supplemented with NAA (0.2 mg/L) in addition to spermidine (100 mg/ L) were recorded the highest significant values of root formation (100 %), roots no.(2.67) and root length (5.42 cm), total soluble phenols (5.94 mg/g f.wt) and AL) activity (124.66 nkat /g protein) as compared to the other studied concentrations treatments. Srivastava, (2002) reported the 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 they 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. It could be noticed that high spermidine concentration had no beneficial effect on root growth characters and chemical analysis. This could be act as a reflect to the balance between its indigenous concentration and the exogenous concentration added . In his concern, Handa and Mattoo (2010) reported that Biogenic amines putrescine, spermidine and spermine are ubiquitous in nature and have interested researchers because they are essential for cell division and viability, and due to a large body of their pharmacological effects on growth and development inmost living cells. In addition, 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. However, AL-Mayahi, (2014) Reported on date palm cv. Ashgar that rooting medium consisting of N6 medium supplemented with 0.2 mg L-1 NAA, copper sulphate and cobalt chloride both at 0.5 µM. Resulted in maximum induction of roots . Total phenol content increased at high concentration of Cu and Co.

5- Acclimatization of plantlets using different growing mixture types:

Data presented in Table (5) and Fig. (1-h) showed that, growing mixture containing compost and perlite (1:1, v/v) recorded the highest significant percentage values of plantlets survival (73.33 %) as well as higher leaves no. (4.7) and leaf area (23.8 cm2) as compared with the other studied growing mixture types. However, compost and rice shell (1:1, v/v) were recorded the lowest results in plantlets survival (43.33), leaves no. (3.2) and leaf area (17.4 cm2). In this regard, (Hegazy, 2008) suggested that the superiority of compost and perlite could be ascribed to their effects

on sparring more suitable conditions for the 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.

6- Acclimatization with using specific pre-treatments for hardening

Results in Table (6) and Fig. (1-i) indicated that plantlets produced in 4 weeks on solid rooting medium and transfer for another 4 weeks to liquid 1/2 salt strength as well as received natural light intensity 8000 Lux in the greenhouse resulted in significant saplings survival (90 %) as compared with the control.

However, no significance could be obtained for leaves no. (4.9) and leaf area (27.11 cm2). Result obtained positive response to hardening and this may due to the good chance for roots to grow in liquid medium and avoid fine roots to destroyed during transfer in acclimatization if it is on solid medium as well as expose to natural light in the green house for 4 weeks could be help in transfer from heterotrophy to autotrophy. Moreover, reduce the transpiration as results to increase the wax film and improve stomata mechanism.

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Tables

Table1: Effect of right timing to induce shoot tip poking and incisions on fresh wt. (g), degree of oxidative browning as well as crude protein, total amino acids, total hydrolysable carbohydrates, total soluble phenols and phenylalanine ammonialyase (PAL) activity on Date palm cv. Ekhlass grown in vitro.

Treatment		Growth characters			Chemical analysis				
Free hormone Medium (MMS)	No. of weeks	Initial Fresh wt.. (g)	Final fresh Wt. (g)	Degree of browning	Total Amino Acids (%)	Crude Protein (%)	Total Soluble carbohydrates (%)	Total soluble phenol (mg / g f. wt.)	PAL activity (nkat /g protein)
Poking & incisions	0	0.98^{a}	1.83^{b}	2.2^{b}	4.11^{b}	23.21^{b}	16.33^{b}	3.15^{b}	87.02^{b}
	2	0.91^{b}	1.71^{c}	1.0^{c}	5.92^{a}	37.31^{a}	19.41^{a}	4.95^{a}	112.12^{a}
	4	0.93^{ab}	1.98^{a}	2.7^{a}	2.87^{c}	17.90^{c}	13.11^{c}	0.78^{c}	57.24^{c}

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

Table 2: Effect of plant growth regulators on fresh wt. (g) and axillary bud proliferation as well as total soluble phenols and phenylalanine ammonialyase (PAL) activity of date palm shoot tips ‘’ cultured in vitro for 12 weeks.

Treatment (mg/L)	Growth characters			Chemical analysis	
	Initial fresh wt. (g)	Final fresh wt. (g)	Axillary Bud growth %	Total soluble phenol (mg/g f.wt)	PAL activity (nkat /g protein)
MMS Free hormone (control)	0.93^{bc}	4.44^{d}	00.00^{b}	2.96^{d}	81.23^{d}
NOA (0.5) + BA	0.88^{d}	12.28^{b}	00.00^{b}	3.47^{c}	86.00^{c}
+ Kin	0.99^{a}	9.48^{c}	00.00^{b}	3.73^{c}	89.06^{c}
+ 2iP	0.95^{b}	10.52^{c}	00.00^{b}	4.17^{b}	94.15^{b}
+ BA (1) + Kin (1) + 2iP (1) + NOA (0.5)	0.92^{c}	17.13^{a}	33.33^{a}	4.77^{a}	99.47^{a}

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

Table 3: effect of growth regulators in addition to putrescine and adenine hemi-sulphate Concentrations on axillary buds multiplication rate, fresh weight (g) and growth value of date palm ‘’ Ekhlass ‘’ cultured in vitro for 14 weeks in dark followed by 8 weeks under light conditions.

Treatments (mg/L)				Growth characters			
Growth Regulators (mg/L)		Putrescine	adenine hemi-sulphate	Axillary buds			
				Formation %	Multiplication Rate	Fresh weight (g)	Growth Value
2iP + Kin + IAA	0.2	0	80	0.00[b]	1.00[b]	1.20[e]	38.5d
		50	80	0.00[b]	1.00[b]	8.22[d]	216.5cd
		100	80	0.00[b]	1.00[b]	21.53[b]	539.5ab
		0	160	0.00[b]	1.00[b]	12.72[c]	396.5bc
		50	160	33.33[a]	2.11[a]	25.20[a]	727.0a
		100	160	0.00	1.00[b]	24.45[a]	602.5ab

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

Table 4: Effects of NAA and spermidine concentrations on root growth characters and chemical analysis of date palm ‘’Ekhlass’’ shoots cultured in vitro for 4 weeks.

Treatment (mg/L)		Growth characters of roots			Chemical analysis	
NAA	Spermidine	Formation %	No.	Length (cm)	Total soluble phenol (mg / g f. wt)	PAL activity (nkat /g protein
Free hormone (control)		22.22 [c]	0.11 [c]	0.44 [c]	4.91c	59.97c
0.2	50	55.56 [b]	0.22 [c]	0.72 [c]	5.42b	108.28b
	100	100.00[a]	2.67 [a]	5.42 [a]	5.94a	124.66a
	150	66.67 [b]	1.22 [b]	2.56 [b]	5.43b	61.23c

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

Table 5: Effect of growing mixture types on survival percentage, number of leaves and leaf length of hardened Ekhlass plantlets after 3 months in acclimatization.

Treatments			Growth characters		
			Survival %	Leaves	
				No.	area (cm^2)
Compost	+ Bark chips	(1:1, v/v)	66.67^{bc}	4.2^{b}	22.5^{b}
	+Perlite	(1:1, v/v)	73.33^{a}	4.7^{a}	23.8^{a}
	+Coconut shell	(1:1, v/v)	50.00 ^{c}	3.8^{c}	20.1^{c}
	+Rice shell	(1:1, v/v)	43.33 ^{b}	3.2^{d}	14.7^{d}

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

Table 6: Effects of pre-acclimatization treatment on survival %, no. of leaves and leaves area (cm2) of date palm ‘’Ekhlass’’ shoots cultured in vitro

Treatments							
Pre-acclimatization on rooting medium				Acclimatization	Survival %	Leaves	
Solid full strength 4 weeks	1/2 salt Strength 4 weeks	Artificial light 1500 Lux	Natural light 8000 Lux	Compost + Perlite		No.	Area (cm^2)
+	-	+	-		73.33^{b}	4.7^{a}	23.80^{a}
+	+	-	+		90.00^{a}	4.9^{a}	24.11^{a}

Figures

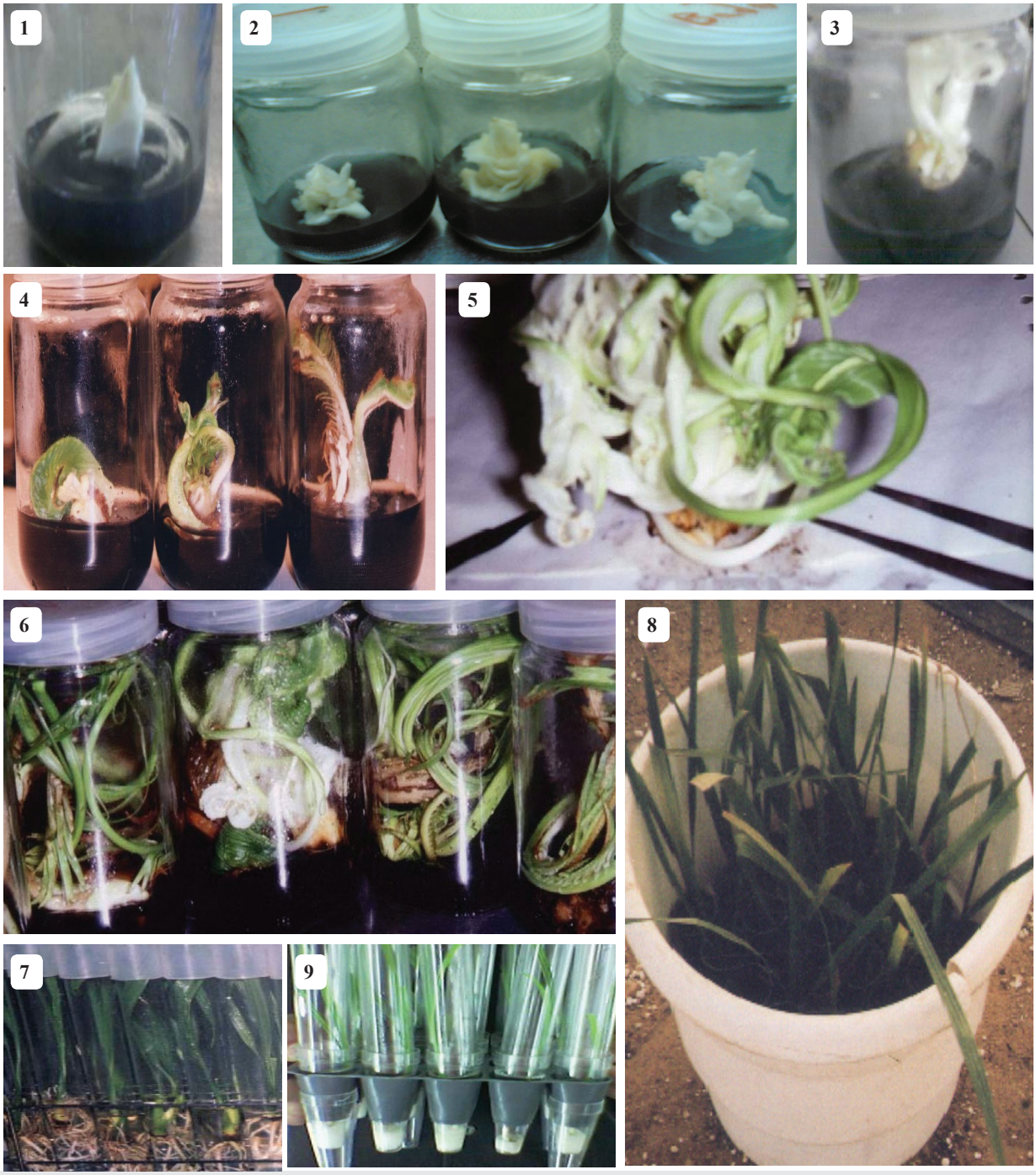

Fig.1. Complete promising protocol for *in vitro* direct organogenesis of date palm cv. Ekhlass a-Shoot tip explant after 2 weeks *in vitro*. b- Shoot tips growing after poking and incisions under dark condition c- shoot tip growth after 3 months and before transfer to light d- Shoot tips growing under light condition e- Shoot tip proliferation after 6 months f- Proliferated shoots growth in elongation stage. g- shoots formed well roots after a month h- Plantlets in pre-acclimatization in liquid 1/2 MS salts strength for a month. i- Healthy plantlets after 3 months in acclimatization.

Effect of different growth regulators on production of somatic embryos from immature inflorescence of *Phoenix dactylifera*

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ABSTRACT

Immature inflorescences of date palm (Phoenix dactylifer) cv. Sewi were used as explants for in vitro culture to investigate the effect of plant growth regulators on inflorescence proliferation. The inflorescences on initiation stage cultured on solidified Murashige and Skoog (MS) basal medium supplemented with Picloram 1, 5, 10 and 15 mg l-1 for two re-cultures. Then, transferred into medium supplemented with different concentration of TDZ combined with NAA on proliferate stage. The optimal concentration for successful inflorescence growth was 5 or 10 mg l-1 Picloram and through studying the residuals effect of Picloram on inflorescences proliferation in the presence of three concentration of TDZ, it found that, 0.5 mg l-1 TDZ combined with 0.1 mg l-1 NAA was more effective to induce direct somatic embryos and gave the highest inflorescence proliferation percentage, while the high level of Picloram induced callus. Vegetative shoots formed into media containing 0.1 NAA mg l-1, 0.2 mg l-1 BA and 0.2 mg l-1 kin. All somatic embryos were converted successfully to healthy normal plantlets which could be transferred to greenhouse.

Keywords: *Phoenix dactylifera*, *in vitro* inflorescences, direct somatic embryos, Picloram, TDZ

Abbreviation: Picloram *(4-amino-3,5,6-trichloropicolinic acid)*,Thidiazuron (*N*-phenyl-*N*'-1,2,3-thiadiazol-5-ylurea),NAA(naphthaleneacetic acid), BA (6-Benzylaminopurine), kin (kinetin).

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world and is mentioned in the Holy Qur'an and Bible. Date palm is one of the most important fruit trees in the Middle East and in the Saharan and Sub-Saharan regions of Africa. In some areas, this is the only tree which provides food, shelter and fuel to the communities. Dates are not only a staple food but are also an important export cash crop) Zaid and Hegarty 2006). Conventional propagation is by offshoots making it slow to establish new date palm plantations. Moreover, several genotypes do not produce offshoots while others are difficult to root. In addition seed-propagated palms do not bear true to type due to heterozygosis and require up to 7 years reaching fruiting stage. The need for rapid and efficient vegetative propagation systems for elite genotypes has therefore become urgent. Since 1970 intensive efforts have been undertaken into large-scale micropropagation of date palm using techniques such as somatic embryogenesis and organogenesis (Drira 1983; Drira and Benbadis 1985; Tisserat 1979; ElHadrami *et al*. 1998).

Exogenously supplied plant growth regulators are essential for somatic embryogenesis (Ammirato 1983). Most tissue culture studies of palms have focused on the effects of different auxin types and concentrations on various explants cultures as investigated in date palm (Othmani et al. 2009, Eke et al. 2005), macaw palm (Moura et al. 2009),

and coconut (Verdeil *et al.,* 1989and Verdeil *et al.,*1994). Picloram has recently been reported to be successful in Arecanut palm tissue culture in terms of callus and somatic embryo production. The continuous production of embryonic calli from the initial explants indicates the potential of the protocol for multiplication of palms (Karun et al. 2004).

Thidiazuron has been used successfully *in vitro* to induce adventitious shoot formation and to promote axillary shoot proliferation (Chin-Yi Lu, 1993).The ability of TDZ to stimulate cell division has been demonstrated in soybean callus.Apart from stimulating cell division, TDZ had also been shown to induce adventitious shoot formation from tobacco leaf discs and to stimulate radish cotyledon expansion (Thomas and Katterman, 1986).

The objective of this study was to investigate inflorescence proliferation of *Phoenix dactylifera* cv. sewi maintained in the immature phase and induced somatic embryos directly. We also systematically examined the residual effects of picloram and the change composition of media to another type of growth regulators like thidiazuron (TDZ) and naphthalene acetic acid (NAA) on proliferation percentage.

MATERIALS AND METHODS

Plant material

Two distinct steps were followed:

The first step had been done outside the laminar flow hood and before cutting the spathes, they were sprayed with 70% ethanol and burned for a few seconds to burn the external hairs. The second step took place under aseptic condition into laminar air flow hood. The spathes were gently opened with sterilized scalpel then the spikelets were soaked in mercuric chloride (Hg Cl2) at 0.1% for 15 min. The explants of inflorescences were rinsed three times with sterilized distilled water.

Spikelet of length (7-10 cm) were cut into 1-3 cm long pieces which each piece carries many florets (Fig.1a,b) Sidky and Eldawyati, 2012)

Inflorescence Initiation (first stage)

To establish direct somatic of *Phoenix dactylifera* cv. Sewi, we first cultured of Spikelets explants of length (7-10 cm) on half macro and full micro elements of Murashig and Skoog (1962) combined with 40 mg-1 adenine–Sulphate, 5mg-1 thiamin- HCl, 100 mg-l myoinositol, 200 mg-1 glutamine, 0.5 g-l activated charcoal, 50g-1 sucrose and 5.0 g-1 agar with different concentration of Picloram 1,5,10, and 15 mg l-1 . Effects of treatments were evaluated after two re-cultures (12 weeks). Data collection and re-culturing were performed at 6 weeks intervals. pH of each medium was adjusted to 5.7 ± 0.1 prior to addition of agar, the medium were distributed into culture small jars (150 ml), the jars were autoclaved at 121°C and 1.2 kg/cm² for 20 min. Culture of all treatments was incubated under complete darkness at 27± $2C^0$. Data were taken on swelling and browning rate.

Inflorescence proliferation (second stage)

In order to examine the effects of plant growth regulators on inflorescence proliferation, we added TDZ at 0.1, 0.5 and 1.0 mg l-1 combined with 0.1mg l-1 NAA to half MS basal medium the explants re-cultured every 6 weeks for twice. As soon as somatic embryos were germinated, it was transferred into MS solid medium containing 0.1 NAA mg/l + 0.2 mg/l BA+0.2 mg/l kin, 200 mg l-1 KH2PO4, 40 g l-1 sucrose and .0.3g l-1 AC (Sidky and Gadalla 2013). Plantlets were transferred to rooting media. Data were calculated after each culture as follows:

1. Number of direct somatic embryos.
2. Percentage of direct somatic embryos.
3. Callus initiation degree/explant.

(This data Scored visually according to Pottino (1981) as follow:

- Negative results (-) 1
- Average results (+) 2
- Good results (+++) 3

Rooting stage

Plantlets were cultured on 1/2 MS liquid medium supplemented with 1.0 NAA mg l-1, 200 mg l-1 mg/l KH2PO4, 40 g l-1 sucrose, 100 mg l-1 myoinsitol and 1g l-1 AC, and incubated under 6000 lux light(Fig.1g), then rooting and eventually successfully transplanted in the greenhouse.

Statistical analysis

The randomized factorial design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% according to Snedecor and Cochran (1980).

RESULT AND DISCUSSIONS

Effects of picloram on Inflorescence Initiation

The immature flower buds were swelling after 6 weeks, some culture turned brown, but several of them enlarged and gave different response (Table 1 and Fig 1c). This culture had to be re-cultured after another 6 weeks to promote further growth. The highest swelling rate (73.33%) was obtained at 5 mg l-1 picloram concentrations. However; this is not statistically significant at 15 mg l-1 picloram (33.33%). Zimmerman, (1993) reported that the pro embryogenic callus were containing auxins to synthesize all the necessary genes to complete the globular stage. However, the auxins

were removed from the culture to make inactive genes or synthesize new gene products for the completion of embryo development. Kawahara and Komamine (1995) reported that, the exogenous auxins were involved in gene expression of early stages of somatic embryogenesis.

Similarly, browning rate differed according to picloram concentrations and ranged from 20.00% to 46.66%, the lowest browning rate was obtained at 5 mg.l-1 picloram concentration. While, 46.66% of these culture turned brown at1mg l-1picloram. 2, 4-D at levels higher than 30 x 10M inhibited callusing and enhanced browning of coconut embryos (Karunaratne and Periyapperuma 1989*)*.

Effects of TDZ on inflorescence proliferation.

Three concentrations of TDZ were tested combined with 0.1 mg l-1 NAA for their effect on date palm inflorescence proliferation (Table 2 and Fig.1e, f).

Twelve weeks after culture initiation on media containing auxin (Fig. 1d), inflorescence buds transferring into media containing cytokinin, Zimmerman, (1993) reported that the pro embryogenic callus were containing auxins to synthesize all the necessary genes to complete the globular stage. After six weeks we observed growth of structures like globular, the structures proliferation to direct somatic embryos after another six weeks (Fig. 1e,f) . Residual effect of picloram observed in (Table 2). 5mg l-1 or 10 mg l-1 picloram produced the highest direct embryos (4.44, 4.33 embryos / culture). The addition of a cytokinin resulted essential to promote growth of somatic embryos from immature flowers, direct somatic embryos occurred at all TDZ concentrations tested, the highest direct of somatic embryos was obtained after floral buds were cultured on medium containing 0.5 mg l-1 TDZ +0.1 mgl-1 NAA (4.41 embryos /culture).

There was also a significant interaction between TDZ concentrations and picloram concentrations. The interaction between 5mg l-1 picloram and 0.5mg l-1 TDZ+0.1 mgl-1 NAA gave the significant result of direct embryos (7 embryos /culture).These results suggested that changing the medium composition could significantly change the number of direct somatic embryos .This type of response shows similarities with the flower bud-like structures observed by Verron *et a*l. (1995) in the monocot lily of the valley, also in bamboo and ginseng explants, which have been shown to flower and thereof proliferate inflorescences *in vitro* in medium containing TDZ (Lin *et al*. 2003; 2004).

The effects of residual concentrations of picloram on the percentage of proliferation embryos are investigated in (Table 3, Fig.1f). Medium containing 10 mg l-1 picloram gave the maximum proliferation embryos response (84.44%). On the other hand, the highest proliferation embryos were obtained after floral buds were cultured on medium containing 0.5 mg l-1 TDZ (85.83%).

We also showed that the effect of picloram concentrations interaction with TDZ concentrations on proliferation embryos percentage. The highest percentage of proliferation embryos were occurred on medium ranged from 1 mg l-1 to 15 mg l-1 picloram concentrations with medium containing 0.5 mg l-1 TDZ.

The result of the study showed that proliferation embryos percentage, from the immature inflorescence was significantly affected by the changing the medium composition. Picloram concentration residues significantly with another type of growth regulator enhanced embryos proliferation. Lin et al.2006,, conclude that TDZ is essential for inflorescence proliferation. Somatic embryo like structures have been observed on walnut immature cotyledons cultured on woody plant medium containing TDZ (0.11 or 1.1 mg/liter) and 2,4-dichlorophenoxyacetic acid (2,4-D) Neuman *et al*.1988. More recently, somatic embryogenesis was reported in watermelon (Compton and Gray, 1992) and muskmelon (Gray *et al*, 1992), again on medium with TDZ and 2,4-D.

The results showed that effects of picloram concentrations on the callus induction from explants were significantly different in Table 4. We observed some flower buds produced callus, the highest callus induction obtained in the high level of picloram at 15 mg l-1 (1.88 degree/jar). On the other hand, transferring the initial explants to medium containing TDZ at 1.0 mg l-1 often increasing callus (1.66 degree/jar). We also showed that the effect of picloram concentrations interaction with TDZ concentrations on callus degree, the highest callus degree were occurred on medium containing 10 mg l-1 with medium containing 1.0 mg l-1 TDZ(2.00 degree/ jar). Ahmed *et al*.2011 reported that, the callus initiation didn't occur without the growth regulators. Induction of somatic embryos on medium containing picloram has also been reported in many species (Castillo *et al*. 1998; Mendoza and Kaeppler, 2002; Preeti and Kothari, 2004).

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Tables

Table (1): Effect of different Picloram concentrations on the Swelling and Browning rate (%) on the first stage.

Picloram (mg l^{-1})	Swelling%	Browning%
1	53.33	46.66
5	73.33	20.00
10	46.66	40.00
15	33.33	26.66
Mean	51.66	33.33
L.S.D. at 0.05	23.07	17.62

Table (2): Effect of auxin residues on number of direct somatic embryos after transferring to cytokinin concentration.

TDZconcentration+	Previous Treatment Picloram (B)				
0.1 mg l^{-1} NAA (A)	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}	15 mg l^{-1}	Mean
0.1 mg l^{-1}	1.00	1.00	3.66	3.00	2.16
0.5 mg l^{-1}	2.00	7.00	5.33	3.33	4.41
1.0 mg l^{-1}	1.00	5.33	4.00	3.00	3.33
Mean	1.33	4.44	4.33	3.11	
L.S.D. at 0.05	A = 0.68	B = 0.79		AB = 1.38	

Table (3): Effect of auxin residues on proliferation embryos percentage after transferring to cytokinin concentration.

TDZconcentration+	Previous Treatment Picloram (B)				
0.1 mg l^{-1} NAA (A)	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}	15 mg l^{-1}	Mean
0.1 mg l^{-1}	30.00	56.67	76.67	63.33	56.66
0.5 mg l^{-1}	73.33	93.33	93.33	83.33	85.83
1.0 mg l^{-1}	40.00	83.33	83.33	53.33	64.99
Mean	47.77	77.77	84.44	66.66	
L.S.D. at 0.05	(A) = 6.63	(B) = 7.65		AB = 13.26	

Table (4): Effect of auxin residues on callus initiation after transferring to cytokinin treatments

TDZconcentration+	Previous Treatment Picloram (B)				
0.1 mg l^{-1} NAA (A)	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}	15 mg l^{-1}	Mean
0.1 mg l^{-1}	1.00	2.00	1.00	2.33	1.58
0.5 mg l^{-1}	1.33	1.33	1.66	1.66	1.49
1.0 mg l^{-1}	1.66	1.33	2.00	1.66	1.66
Mean	1.33	1.55	1.55	1.88	
L.S.D. at 0.05	(A) = 0.58	(B) = 0.68		AB = 1.17	

Figures

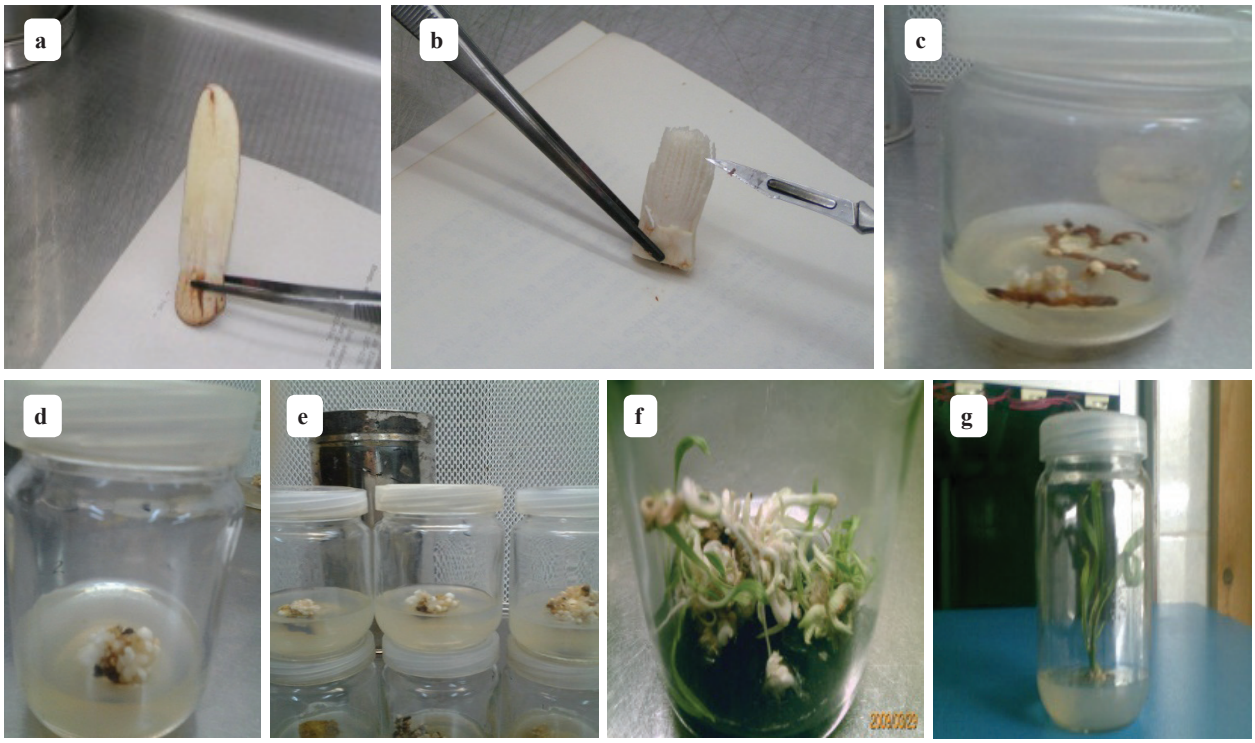

Fig. 1 a) The spathe from 7-10cm, b) Explant after remove the outer protective sheath, c) Swilling of explant, d) Initiation of Explant, e) Flower bud-like structures, f) Explant proliferation to embryos, g) Healthy plantlets

Callus growth and somatic embryogenesis as affected by putrescine and salicylic acid in date palm bream cv.

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ABSTRACT

Different concentrations of putrescine and salicylic acid were examined for their effect on embryonic callus and subsequent embryogenesis in *Phoenix dactylifera* cultivar Bream. Shoot tips were excised from 2-3 years old offshoots, surface sterilized and inoculated onto Murashiege and Skoog, 1962 (MS) medium supplemented with 50 mg/L picloram and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was obtained after 24 weeks on the nutrient medium. Calli were then transferred onto fresh MS medium containing 0.0, 0.5, 1.0, 2.0 or 3.0 mM of putrescine or salicylic acid individually. Results were recorded after 12 weeks. A significant increase in embryonic callus fresh and dry weights was recorded reached 2.3 and 0.3 g respectively at 2.0 mM of putrescine and 5.0, 0.27 g at 3.0 mM of salicylic acid. After inoculation of such embryonic callus onto a fresh medium containing the same concentrations of putrescine or salicylic acid, number of mature embryos increased up to 10.3 achieving 1.7 g fresh weight for ten embryos at the concentration of 3.0 mM putrescine. Number of embryos reached 10.0 with a mean fresh weight reached 1.7 g for ten embryos at 0.5 mM of salicylic acid. It is concluded that both putrescine and salicylic acid may play a positive role in increasing callus growth and regulation of somatic embryogenesis in *Phoenix dactylifera* var. Bream tissue cultures.

Key words: *Phoenix dactylifera* L. embryos, *in vitro, r*egeneration, salicylic acid, putrecine

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) (2n=2x=36) is a dioeciously, perennial, monocotyledon fruit trees belong to the family of Arecaceae (Barrow, 1998). Dates are the major fruit crop of arid climate region in Middle East and North Africa. The heterozygosis of date palm makes its progeny strongly heterogeneous (Munier, 1981). Thus the propagation of date palm through offshoots is preferred over the seedlings. Since propagation through offshoots is slow and affected by their low survival rate, tissue culture of female plants has been preferred widely for mass production of true-to-type plants of elite varieties in demand.

Since the first attempts of date palm propagation via tissue culture that proposed by Schroeder (1970) and Reuveni et al. (1972) until now, two methods of propagation were developed, somatic embryogenesis and direct organogenesis. The production of somatic embryos from embryogenic callus was reported by many researchers (Reuveni, 1979; Mater, 1983; Omar, 1988 and Al Musawi, 2001) as well as from axillary branching of shoot tips (Tisserat, 1991 and Hameed, 2001). While Al-Maari, and Al Ghamdi (1997) and Al Khateeb et al. (2002) succeeded in enhancing adventitious bud formation on shoot tips. The first is the most common micropropagation method in commercial plant tissue culture labs.

Recently, Ibrahim (2012) initiated callus after the addition of 50 mg/L Picloram and 3.0 mg/L 2iP, re-cultured at 4 week intervals until transfer to embryogenic callus proliferation medium. The medium composition varies from researcher to another, for example it consisted of MS salts and vitamins without hormone (Omar 1992) or the same supplements described for callus induction medium (Jasim and Saad 2001; Ibrahim, 2012) for eight weeks. Embryogenic calli are transferred to hormone free MS medium (Omar, 1992) or supplemented with 0.1 NAA (Jasim and Saad 2001). The low rate of asexual embryo formation and germination prompted many researchers to enhance the processes.

It was found that supplementing the culture medium with 1 g/L apple seed powder (Saleh et al. 2006), 25 g/L corn seed powder (Jasim et al. 2008), 100 mg/L vitamin E (Al-Meer and Al-Ibresam, 2010), and 20% (v:v) coconut water or casein hydrolysate at 2.0 g/L (Khierallah and Hussein 2013). All these treatments increased somatic embryogenesis and germination percentages for several Iraqi date palm cultivars.

Mature or germinating embryos initiated roots on a medium consisted of MS salts plus 0.1 NAA and 0.01 BA (Omar *et al.,* 1992). Jasim and saad (2001) used half strength MS salts, 0.1 mg/L NAA, 30g/L sucrose, and 3g/L activated charcoal to improve rooting in Barhi cv. This stimulates root induction and shoots elongation which led to full growth plantlets having about 5-cm-long shoots. Maintenance of these plantlets until they reached 8 to 10 cm long increased their survival rate in soil. Jain et al. (2011) indicated that embryogenic cell suspension cultures have shown higher morphogenesis capacity when compared with other in vitro methods.

Putrescine, or tetramethylenediamine, is an organic chemical compound NH2(CH2)4NH2 (1,4-diaminobutane or butanediamine) that is related to cadaverine; both are produced by the breakdown of amino acids in living and dead organisms. It is found that putrescine enhances growth and morphogenesis in plant tissue culture (Goerge *et al.,* 2008). Hegazy and Aboshama (2010) suggested an efficient novel pathway in date palm micropropagation protocol by induction of direct somatic embryogenesis from bud tissues through putrescine incorporation into the culture medium at 150 mg/L plus a cretin combination of plant growth regulators. They also recorded the highest multiplication rate and growth of date palm embryos by adding 100 mg/L putrescine.

Salicylic acid is a monohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid. It is widely used in organic synthesis and its function as a plant hormone. It appears to have a role in systemic acquired resistance to pathogens and is able to induce various pathogen resistance proteins (Goerge *et al.,* 2008).

Therefore the aim of this study is to examine various concentrations of putrescine and salicylic acid on enhancing embryonic callus and subsequent embryogenesis in *Phoenix dactylifera* Bream cv.

MATERIALS AND METHODS

Young offshoots of Bream cultivar (2-3 years old) were chosen and detached from mother palm. Leaves were dissected acropetaly. Shoot tips of 3 cm in length (apical meristem with soft inner leaves), were excised along with immature fiber of 2 cm in diameter. Explants were dipped in antioxidant solution consisted of 150 mg/L citric acid plus 100 mg/L ascorbic acid (Tisserat, 1991). Explants were surface sterilized with 2.0% sodium hypochlorite solution containing eight drops of Tween-20 as emulsifier for 20 minutes under vacuum, and rinsed three times with sterile distilled water. They transferred to Petri dishes where leaf primordia were removed except the two pairs surrounding the apical meristem which then divided longitudinally into four equal segments and cultured in jars aseptically. The medium of initiation stage was composed of Murashige and Skoog (1962) (MS) salts plus the following (in mg/L); thiamine-HC1 1.0; pyridoxine-HCl 1.0; adenine sulfate.2H2O 40; myo-inositol 100; NaH2PO4.2H2O 170; sucrose 30000 activated charcoal 2000 and agar-agar 7000. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or HC1, before the addition of agar. The medium was dispensed into culture jars with aliquots of 25 ml in each, then covered with polypropylene caps and autoclaved under 1.04 kg/cm² at 121 °C for 15 minutes. Callus initiation medium was supplemented with 50 mg/L picloram and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was obtained after 24 weeks of growth in full darkness.

Calli were then transferred onto fresh MS medium containing 0.0, 0.5, 1.0, 2.0 or 3.0 mM of putrescine or salicylic acid individually. Cultures were incubated in a growth room under low light intensity of 1000 lux for 16 hours daily at 27±1 °C for four weeks. Results of callus fresh and dry weights, number of germinated embryos and mean fresh weight of ten embryos were recorded after 12 weeks.

Experiments were conducted as factorial using Complete Randomized Design (CRD), with ten replicates. Least significant differences (LSD) were used to compare means at 5% level probability.

RESULTS

Supplementation of the callus initiation medium with putrescine (table 1) exhibited a significant increase in both fresh and dry weights at the concentration 2.0 mM of putrescine while other levels, although increased the weights but not up to a significant level.

Addition of salicylic acid (table 2) as a supplement to the embryonic callus medium at concentrations 2.0 and 3.0 mm led to a significant increase in callus fresh and dry weights reached 5.0, 5.0 and 0.25, 0.27 respectively.

The number of formed embryos increased proportionally after the inclusion of putrescine to the medium till reached to a significant level at the concentrations 2.0 and 3.0 mM recording 9.1 and 10.3 embryos respectively. However, mean fresh weights for these emerged embryos fluctuated with a maximum weight reached 1.70 g after inclusion of 0.5 mM of putrescine. All concentrations of putrescine caused no significant differences in the mean of 10 embryos fresh weights compared with those grown on putrescine free medium.

Table 4 shows that all levels of salicylic acid resulted in a significant increase in number of formed embryos compared with those initiated on a medium lacking salicylic acid. Number of embryos was doubled at the concentration 2.0 mM and continued increasing up to 10.6 embryos at 3.0 mM of salicylic acid. These increments in number of embryos although led to increasing the mean fresh weight of 10 emerging embryos, but the highest was recorded at 1.0 mM of salicylic acid recording 2.40 g.

DISCUSSION

Since propagation via somatic embryogenesis has become a vital mean for propagating many plant species, optimization of the culture medium may require some supplements other than plant growth regulators which are normally added to the nutrient medium. It is clear from the reported data (tables 1 & 2) that putrescine and salicylic acid has increased callus fresh and dry weights when supplemented to the callus maintenance medium separately. Putrescine is a polyamine with low molecular weight. It has been implicated in many cellular processes such as cell division, protein synthesis and DNA replication. The recent work of Ravindra and Nataraja (2013) reported that putrescine enhances callus growth, somatic embryogenesis and plant regeneration in many plant species including *Pinus gerardiana* at a concentration of 2.0 mg/L, however it is a genotype dependent. Hossein et al. (2011) speculated that the capability of a certain plant tissue to onset embryogenesis differs with regard to media composition, genotype, organ ontology and stage of differentiation. The results of their studies unequivocally suggest that, irrespective of the type of explants and media culture, salicylic acid increments beyond than 75 µM negatively affect somatic embryogenesis in carrots.

This study has proved (tables 3&4) that putrescine and salicylic acid have improved embryogenesis represented by almost doubling the number of embryos when both were supplemented individually at concentrations exceeded 2 mM. They may trigger competent cells to form cell aggregates which then developed to embryos. Our results are in agreement with those Lewis et al. (2003) who reported that putrescine improves embryogenesis in cotton plants and disagree with those of Husseini et al (2011) who reported that high concentrations of salicylic acid inhibit somatic embryogenesis in carrot tissue cultures. The doubling in embryo numbers obtained after the addition of putrescine or salicylic acid has accompanied by a decrease in embryo weights as a result of the competition on nutrients available in the culture medium. It is concluded from the current study that inclusion of 2-3 mM of putrescine or salicylic acid to the embryonic date palm culture medium has improved somatic embryogenesis. The finding can be exploited commercially by date palm micro-propagators to almost double their production. Searching for other media supplements with the aim of increasing embryo numbers and weights is a vital aspect and requires intensive investigation.

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Tables

Table 1: Callus fresh and dry weights initiated on MS medium supplemented with 50 mg/L picloram and 3 g/L 2iP at different concentrations of putrescine for 24 weeks.

Putrescine (mM)	Callus fresh wt (g)	Callus dry wt (g)
0	1.16	0.05
0.5	1.4	0.07
1	1.85	0.08
2	2.3	0.3
3	1.79	0.1
LSD 0.05	0.759	0.094

Table 2: Callus fresh and dry weights initiated on MS medium supplemented with 50 mg/L of picloram and 3 g/L of 2iP at different concentrations of salicylic acid for 24 weeks.

Salicylic acid (mM)	Callus fwt (g)	Callus dwt (g)
0	1.16	0.05
0.5	1.67	0.09
1	1.92	0.07
2	5	0.25
3	5	0.27
LSD 0.05	1.956	0.105

Table 3: Mean number of germinating embryos and mean weight of ten embryos initiated from calli after supplementation with different concentrations of putrescine.

Putrescine (mM)	No. of embryos	Mean wt 10 embryos (g)
0	4.3	1.36
0.5	7.6	1.7
1	7.5	1.52
2	9.1	1.45
3	10.3	0.96
LSD 0.05	3.663	0.529

Table 4: Mean number of germinating embryos and mean fresh weight of ten embryos initiated from calli after supplementation with different concentrations of salicylic acid.

Salicylic acid (mM)	No. of embryos	Mean wt 10 embryos (g)
0	4.3	1.36
0.5	10.1	1.85
1	7.5	2.4
2	8.6	1.33
3	10.6	1.79
LSD 0.05	2.719	0.702

Multiplication and germination of somatic embryos obtained from cell suspensions of date palm (*Phoenix dactylifera*)

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ABSTRACT

Establishment and development of embryogenic suspension cultures in date palm (*Phoenix dactylifera* L.) cultivars, namely Bouskri (BSK) was implemented using liquid medium with BAP (0.3mg/l) and 2, 4-D (0.1mg/l). Detailed morphological observations have revealed that the cells destined to become somatic embryos divided into spherical proembryos (globular stage) within 7-15 days, with subsequent conversion globular stage to elongation stage after 17 days and cotyledonary stage after 27 days of suspension cultures.

Effects of activated charcoal and glutamine in production of somatic embryos were studied. Activated charcoal (AC) used at 0.15%, improved growth rate somatic embryos and decreased tissue and medium browning, charcoal has significantly reduced phenolics and peroxidase activity in comparison with medium without charcoal. Addition of gultamine (100 mg/l) to the somatic embryo culture medium led to decreased peroxidase activity and increased proteins content in comparison with medium without glutamine.

Somatic embryos were conducted on MS liquid medium diluted a half without plant growth regulator and transferred after on MS solid medium led to improvement of the germination rate to (32%).

Key words: Date palm; somatic embryogenesis; suspension culture; activated charcoal; glutamine; phenolics; peroxidases.

1-INTRODUCTION:

Somatic embryos have been used as a model system to understand the mechanisms regulating plant embryogenesis, being an alternative for the propagation of plants with high rates of multiplication, with relevance in tree improvement programs (Anjaneyulu *et al.,* 2004). In the case of date palm, a regeneration protocol *via* somatic embryogenesis in liquid medium has been established (Fki *et al.,* 2003; Zouine and El Hadrami 2007; Abohatem *et al.,* 2011). This protocol allows the production of a large number of individual somatic embryos (SE) of uniform physiological and growth characteristics and with synchronized development (Abohatem *et al.,* 2011).

During the somatic embryogenesis in date palm (*P. dactylifera*), AC had been employed in every stage. For callogenesis and embryogenesis 0.15 g/l was employed. For embryo maturation and germination 0.25 g/l were used along with MS medium (Zouine *et al.,* 2005). AC induced somatic embryogenesis was also reported in *Phoenix dactylifera* L. by Fki *et al*. (2003), where the presence of 0.3 g/l AC in liquid medium resulted in the differentiation of large number of SEs.

Amino acid enrichment of the culture medium increased the number of regenerated embryos as well as storage protein accumulation and their conversion rate into vitro plantlets in the case of alfalfa (Lai *et al.,* 1992; Stuart *et al.,* 1985). Glutamine alone or in combination with casein hydrolysate and inorganic forms of nitrogen, has generally been used in the different phases of somatic embryogenesis (Fki, 1998; Morcillo *et al.,* 1999; Garin *et al.,* 2000).

The objectives of this study were (i) to describe morphological characteristics of somatic embryogenesis at different development stages from the cell suspension culture (ii) to compare the effect of activated charcoal and glutamine on embryogenic cells in suspension cultures on the basis of their phenolic contents, peroxidase

activities and protein contents and (iii) to improve germination rate of date palm somatic embryos

2-MATERIAL AND METHODS

2-1. Establishment of cell suspension:

To establish the cell suspension, the method described by Fki *et al.* (2003), and Zouine and El Hadrami, (2007) has been used in this study. Five hundred milligrams of embryogenic callus (granular aspect with globular embryos) were cut with sterile scalpel into small pieces (fine parts) as possible and then transferred in 50 ml of liquid medium in 250 ml Erlenmeyer. The content of Erlenmeyer is passed through sieves with a 500 µm mesh size and the filtrate is incubated on a rotary shaker (100 rpm) at 25 ± 2ºC under a 16/8-h (light/dark) photoperiod. The liquid medium is that MS/2 supplemented with 2,4-D (0.1 mg/ l), BAP (0.3 mg /l) (Abohatem *et al*. 2011).

Two factors (Activated charcoal and Glutamine) were tested in the culture medium which supplemented with 150 mg/l AC and without AC or supplemented with 100mg/l Glutamine and without G.

2.2. Development of somatic embryos in cell suspension culture:

The development and division of embryogenic cell during 15 days from cell suspension culture was observed under a microscope. Morphological characteristics of somatic embryogenesis at different developmental stages (globular, elongation and cotyledonary) were recorded from the cell suspension culture during the induction of somatic embryogenesis.

2.3. Maturation and germination of somatic embryos:

Maturation and germination of somatic embryos are conducted on MS liquid medium diluted a half without plant growth regulator for two weeks. After that somatic embryos are transferred and cultured on MS solid medium supplemented with NAA (0.1 mg/ l).

2.4. Extraction and analysis of phenolics:

Phenolics compounds were extracted and analysed as described by El Hadrami (1995). Fresh somatic embryogenesis tissues (250 mg) was homogenized with 2ml methanol (80%) at 4ºC and centrifuged three times at 7000g for 3 min, supernatants were recuperated each time. 100 µl of the supernatant was added to Folin-Ciocalteu reagent (250 µl) and Sodium carbonate (20%). The mixture was incubated at 40ºC for 30 min and the blue colour was determined at 760 nm.

2.5. Extraction and analysis of proteins:

Total soluble proteins were extracted according to the method described by Lecouteux (1993). Fresh somatic embryogenesis tissues (250 mg) was homogeneneised with 2ml Tris maleate buffer (0.1M, PH 6.5) and centrifuged for 6min at 7000g. The supernatant was used as the crude proteins extract. The total proteins were measured by spectrophotometer at 595 nm according to the method described by Bradford.

2.6. Peroxidase extraction, activity assays and elctrophoresis:

Somatic embryos tissues (250 mg FW) were homogenized in 1 ml of Tris maleate buffer pH 6.5 (0.1M). After centrifugation at 10 000 *g* for 10 min, the supernatant was used for enzymatic activities determination. Peroxidase (POX) activity was assayed as described by Baaziz et al. (1994).

To separate peroxidase isoenzyme, polyacrylamide gel electrophoresis of soluble proteins was carried out according to Baaziz (1989). For POX staining, the gel was incubated for 15 min in 100 ml of 0.1M sodium acetate buffer pH 5 containing 0.1g of benzidine and 0.1 ml 10% hydrogen peroxide. Gels were incubated with the substrates for 30 min in dark until dark bands appeared.

2.7. Statistical analysis:

Results were analyzed by variance analysis (ANOVA), followed by SNK test at P = 0.05 level to compare means (SPSS, 1996). The number of repetitions is three replicates with two independent experiments.

3. RESULTS AND DISCUSSION

3.1. Development of somatic embryos:

Microscopic observation was carried out to describe the development of embryogenic cell during 15 days from cell suspension culture (Fig.1A-B). During the first week, single cells of suspension cultures divided and formed small clumps of cells (Fig. 1C) whereas after one week, cells were dividing actively and formed large clumps of cells (Fig. 1D).

Similar result was observed in oil palm by Roowi *et al*. (2010) who showed that cell suspensions contained cellular aggregates composing of round cells with dense cytoplasm that were small in diameter (10–20 µM). Kramut and Te-chato (2010) described two types of cell aggregate, 5 to 10 cells and more than 10 cells. For aggregate consisting of more than 10 cells, those cell showed dense cytoplasm whereas 5 to 10 cell aggregate consisted of large vacuolar cells.

The morphological observations of cell suspension cultures have revealed that the cells destined to become somatic

embryos divided into spherical proembryos (globular stage) within 7-15 days (Fig. 1E), with subsequent conversion globular stage to elongated embryos after 17 days (Fig. 1F), cotyledonary embryos (Fig. 1G) were formed in the next 27 days and somatic embryos were formed in the next 35 days (Fig. 1H). To our knowledge, this is the first time description of the time course of date palm embryo formation in a suspension culture.

3.2. Effect of activated charcoal on phenolics, proteins content and peroxidases activities of date palm somatic embryos:

To limit tissue browning that cause an appreciable loss of culture viability, activated charcoal are commonly used in palm tissue culture to trap phenols and oxidized phenols. Activated charcoal used only at 150 mg/l improved growth rate somatic embryos and decreased tissue and medium browning (Fig.2).

The biochemical analysis showed that charcoal was significantly reduced phenolics (0.12 mg/g FW) in comparison with medium without charcoal (0.33 mg/g FW). It reduced peroxidase activity from 69.8 UE/g FW to 35.6 UE/ g FW. In addition, charcoal decreased proteins content (68.9 µg/g FW) in comparison with medium without charcoal (77.84 µg /g FW) (Table 1).

Until the actual time, there was a little information about biochemical parameters implicated in tissue browning in palms and particularly in date palm. The viability of somatic embryo is considered to be one of the most difficult to maintain. Previous works showed that this phenomenon is in relation with the high accumulation of caffeoylshikimic acids in the somatic embryo.

Activated charcoal was found to be the best antibrowning factor particularly during the first months of suspension culture. This result is in good agreement with the already known facts concerning the role of this compound to trap phenols and oxidezed polyphenols in palm tissue culture (Touchet *et al*. 1991; Teixeira *et al.,* 1993, 1994; Verdeil *et al.,* 1994; El Hadrami and Baaziz 1995; El Hadrami *et al.,* 1995; Othmani *et al.,* 2009).

3.3. Effect of gultamine on phenolics, proteins content and peroxidases activities of date palm somatic embryos:

The application of gultamine (100mg/l) to the somatic embryo culture medium led to decreased peroxidase activity (35.6 U E / g FW) in comparison with medium without glutamine (56.8 U E / g FW) and increased proteins content from 75.6 µg / g FW to 87.84 µg / g FW (Table 2).

Zouine and El Hadrami (2007) found that the use of 2, 4-D (0.1 mg/ l) in combination with glutamine (6.7 ×10 - 4M) gives a significant ($P < 0.05$) enhancement in soluble protein and sugars in embryogenic cultures of date palm. In Oil Palm somatic embryos, the utilisation of glutamine alone or in combination with arginine was found to be a factor of enhancement of protein accumulation (Morcillo *et al.,* 1999).

3.4. Effect of Glutamine and activated charcoal on peroxidase isoforms:

When separated by polyacrylamide gel electrophoresis, peroxidase extracts from somatic embryos of the date palm cultivar BSK, showed a major migration zone with Rf value interval 0.10-0.16 (Fig. 3), where the stain intensity increased with the high volume of embryo extract (60 µl).

Extracts with activated charcoal and glutamine were characterized by a peroxidase isoform of relatively high migration speed (Rf = 0.16). This latter diappeared in peroxidase extacts prepared from embryos cultivated on media without activated charcoal and glutamine. This result confirms that AC and G modulate browning by their action on qualitative aspect of peroxidases. They induce the formation of an enzyme isoform, which migrates at Rf = 0.16 on 11% polyacrylamide gels. This isoform correlated with embryo maturation.

3.5. Somatic embryos germination:

When the somatic embryos were conducted on MS liquid medium diluted a half without plant growth regulator and transferred after on MS solid medium supplemented with NAA (0.1 mg/l) an improvement of the germination rate (32%) was obtained. Only 8% of germination was reached when embryo were transferred directly on MS solid medium (Fig.4). Similar result was obtained by Fki et *al.* (2003), where they showed a 25% germination rate of date palm somatic embryos on modified MS medium deprived of plant growth regulator.

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Tables

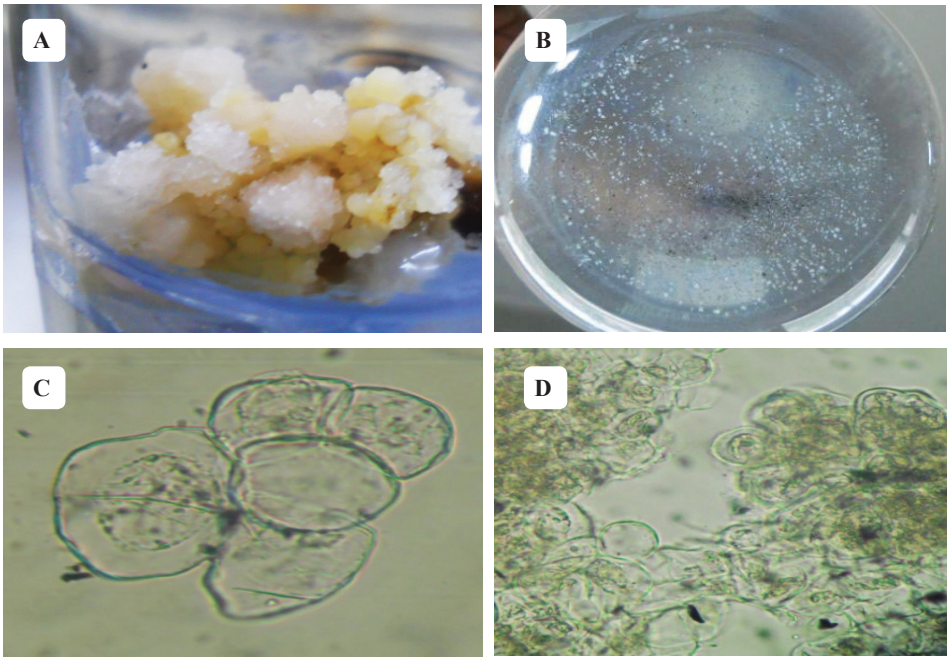
Table 1: Effect of activated charcoal on phenolics, proteins content and peroxidases activities of date palm somatic embryos

Medium	Total protein µg / g FW	Phenols mg / g FW	Peroxidase activity UE / g FW
With charcoal	87.84 ± 3.6	0.12 ± 0.05	35.6±23.2
Without charcoal	77.43 ± 2.8	0.33 ± 0.04	69.8 ± 26.8

Table 2: Effect of glutamine on phenolics, proteins content and peroxidases activities of date palm somatic embryos.

Medium	Total protein µg / g FW	Phenols mg / g FW	Peroxidase activity UE / g FW
With gultamine	87.84 ± 3.6	0.12 ± 0.05	35.6±23.2
Without gultamine	75.6± 4	0.15 ± 0.03	56.8 ± 33.7

Figures



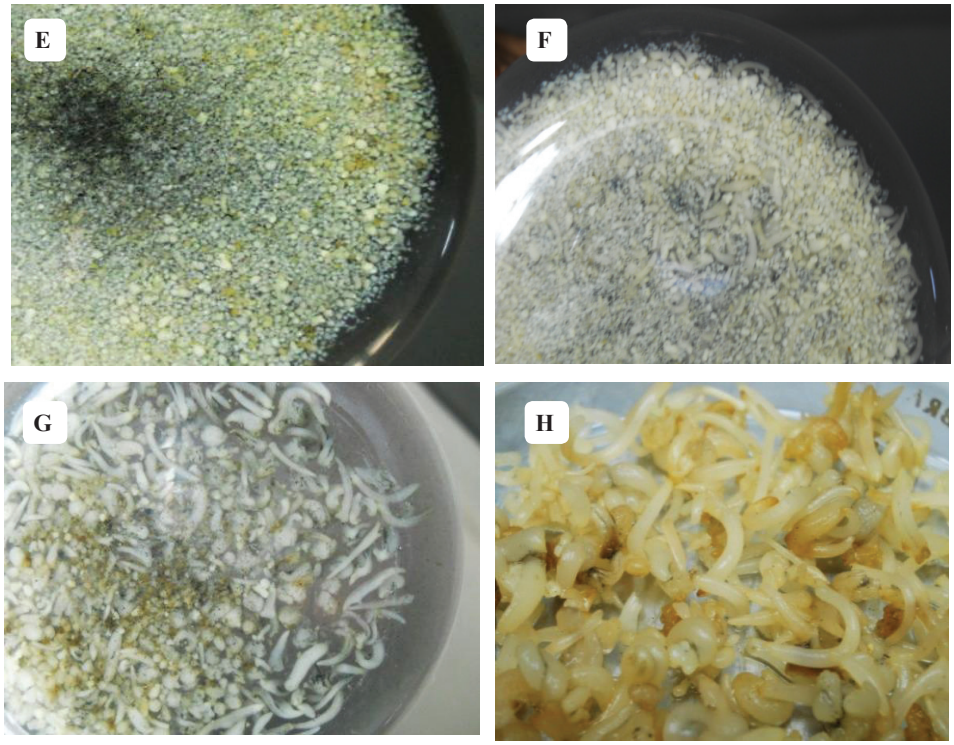


Fig. 1: The morphology of date palm cell suspension culture during the induction of somatic embryogenesis. (A) embryogenic callus, (B) cell suspension after 7 days of culture, (C) small clumps of cells during the first week under ×100 enlargement with light microscope, (D) large clumps of cells during the second week under ×100 enlargement with light microscope, (E) embryogenic cell in globular stage after 14 days of culture, (F) conversion of globular stage to elongated embryos after 21 days of culture, (G) cotyledonary embryos after 32 days of culture, (H) somatic embryo after 40 day of culture. Scale bar: 0.1mm.

Fig. 2: Effect of activated charcoal on phenolics and tissue browning in date palm somatic embryos

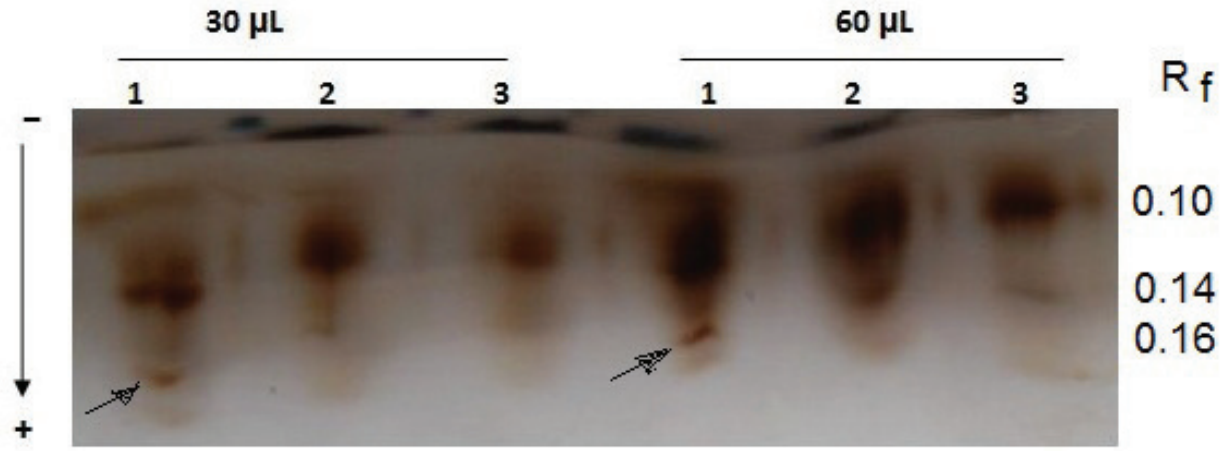


Fig.3: Zymogram of peroxidases extracted from somatic embryos of date palm (cultivar BSK), separated by polyacrylamide gel electrophoresis (11% gels) and revealed with benzidine, as substrate. Extract samples (30 µl and 60 µl) are loaded for cultures 'with AC and G' (1), 'without G' (2) and 'without AC' (3). Arrow indicates peroxidase isoform of Rf 0.16.

Fig. 4: Germination of somatic embryo

Increasing pollination efficiency in saidy date palms by using starch carrier along with pollens suspension

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ABSTRACT

During 2011 and 2012 seasons, Saidy date palms product from offshoot were pollinated with pollens (water suspension pollens) at four levels namely 5.0, 2.5, 1.25 and 0.625 g/ I water with or without starch carrier at the same previous levels. The goal was enhancing pollination efficiency which reflected on promoting fruit setting %, yield and fruit quality of such date palm cv.

It is worth to mention that pollination with water containing pollen grains (0.625 to 5 g/ L water) and starch carrier (at 0.625 to 5.0 g/ I water) was preferable than using water suspension pollens alone in enhancing fruit setting %, yield and fruit quality.

The promotion was associated with increasing pollen levels in water without using starch carrier. This effect was changed with using pollens with starch carrier, since using water suspensions containing 1.25 g pollens/ I water besides 5.0 g starch carrier gave the best results in this respect.

For promoting production of Saidy date palms, it is necessary for carrying out pollination using water suspension containing 1.25 g pollens + 5.0 g starch/ I water.

Key words: Pollens, pollination, efficiency, starch, yield and Saidy date palms.

INTRODUCTION

Research on mechanical pollination started in 1950 . since then several systems for rapidly applying pollen were investigated to overcome these problems, including helicopters, fixed-wing aircraft, ground-level duster and pollen grain suspension sprays .

Hand pollination is the most expensive operation due to climbing several times according to the pattern of flowering for the palms (Al- Baker, 1972; Hussien *et al.,* 1979 and Hussein, 1983). 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 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: and 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 & Mahmoud, 1986; El-Mardi *et al.,* 1995; Hussein and Hassan, 2001; Ragab *et al.,* 2004; Ashour *et al.,* 2004 and El-Salhy *et al.,* 2007).

Mechanization of date production is becoming more and important due to the rising cost of production and shortage of man power, in particular it is difficult to find skilled labor to work during the peak pollination season

Moreover, using of pollen grains mixed with pure water was successful in pollination of saidy date palm cultivar and it was recommended to use 2.5-5g/liter of water in pollination of it (El-Salhy, et al 2010).

This study aimed to innovating an untraditional method in date palm pollination which combined both mechanical pollination and fruit thinning effect in addition to get high yield with good quality.

MATERIALS AND METHODS

This study was conducted in date palm Research Farm in Agricultural Research Station, at El-Kharga Oasis, New Valley Governorate, Egypt, during two successive growing seasons 2011and 2012, on 40 years old saidy date palm cultivar (as semi dry date palm cv.)

Twelve 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 were adjusted to twelve by removing excess earliest, latest and smallest clusters for achieving of the following four treatments:

1. Spraying pollen grains suspension (5 g pollens/L)
2. Spraying pollen grains suspension (5 g pollens/L + 5 g starch/L water).
3. Spraying pollen grains suspension (5 g pollens/L + 2.5g starch/L water).
4. Spraying pollen grains suspension (5 g pollens/L + 1.25 g starch/L water).
5. Spraying pollen grains suspension (5 g pollens/L + 0.625 g starch/L water).
6. Spraying pollen grains suspension (2.5 g pollens/L + 5 g starch/L water).
7. Spraying pollen grains suspension (2.5 g pollens/L + 2.5 g starch/L water).
8. Spraying pollen grains suspension (2.5 g pollens/L + 1.25 g starch/L water).
9. Spraying pollen grains suspension (2.5 g pollens/L + 0.625 g starch/L water).
10. Spraying pollen grains suspension (1.25 g pollens/L + 5 g starch/L water).
11. Spraying pollen grains suspension (1.25 g pollens/L + 2.5 g starch/L water).
12. Spraying pollen grains suspension (1.25 g pollens/L + 1.25 g starch/L water).
13. Spraying pollen grains suspension (1.25 g pollens/L + 0.625 g starch/L water).
14. Spraying pollen grains suspension (0.625 g pollens/L + 5 g starch/L water).
15. Spraying pollen grains suspension (0.625 g pollens/L + 2.5 g starch/L water).
16. Spraying pollen grains suspension (0.625 g pollens/L + 1.25 g starch/L water).
17. Spraying pollen grains suspension (0.625 g pollens/L +0.625 4 g starch/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.

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 liter 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 is removed after four weeks.

Measurements:

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:

$$\text{Fruit set } \% = \frac{\text{Number of fruits setting on the strand}}{\text{Total number of flowers per the strand}} \times 100$$

Yield and fruit quality:

Bunches were harvested at tamr stage (last week of September), fruit weight/bunch (kg) was recorded. Twenty five 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 volumetric method that 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 L.S.D. 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:

Fruits 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 (1) showed the effect of pollination with pollens (water suspension pollens) four levels namely 5.0, 2.5, 1.25 and 0.625 g/ I water with or without starch carrier at the same previous levels on fruit set percentage and fruit weight/bunch of saidy date palm during 2011, and 2012 seasons.

Data showed that there are significant differences in fruit set percentage and fruit weight/bunch due to pollination by using pollen grains suspension (5 g/L) alone (T0) compared with using pollen grains suspension (5 g/L) with starch carrier (5 g) (T1). The fruit set percentage values were (60.45 and 70.39%) whereas, the fruit weight/bunch were (9.98 and 10.73kg) as an av. of two studied seasons

However, there was a reduction on the fruit set percentage and fruit weight/bunch with reducing of the pollen grains suspension concentration, and starch concentration in suspension so, there was a significant decrease in fruit set percentage and bunch weight due to pollination with 0.62 g/L plus 0.62 g starch/L water (T16). compared with 5g/L plus 5 g starch/L water (T1).

These findings could be attributed to the reduction of fruit set as the pollen grains suspension concentration is reduced. in turn This leads to reduce the fruit retention, hence the fruits weight/bunch was reduce. The above mentioned results are in agreement with those obtained by (Hussein et al 1979; Shabana et al1998; Ragab et al 2004and El-Salhy, et al 2010).

It can be concluded from these results that using spraying, it had proved importance from economic point of view . The use of spray treatments reduce the amount of pollen to 0.01 from the amount used by dusting and this dose had insignificant effect on fruit retention or yield .On other hand, The pollination with pollen dust need to centuple of pollen grain amount that pollination as pollen grain suspension spraying. Therefore, pollen grain suspension lead to increase the pollination efficiency, decrease consumption of pollen grains and reduce the pollination costs

Fruit quality:

A-Physical characteristics:

Data in Table (2) clearly showed that there was significant differences in Fruit weight (g) Fruit length (cm) and Fruit diameter (cm) due to pollination by using pollen grains suspension (5 g/L) alone (T0) compared with using pollen grains suspension (5 g/L) with (5 g) starch carrier (T1). The Fruit weight (g) values were (9.84 and 10.52 g) whereas, Fruit length (cm) were (3.63 and 3.69 cm) and Fruit diameter (cm) (2.23 and 2.26) as an av. of two studied seasons These results could be due to the reduction on the fruit set percentage

However, there was an increasing on the fruit physical characteristics with reducing of the pollen grains suspension concentration, and starch concentration in suspension

So, there was a significant increase in fruit physical characteristics due to pollination with 5g/L plus 5 g starch/L water (T1),compared with 0.625 g/L plus 0.625 g starch/L water (T16).

The best results dealt with fruit physical properties is observed on palms pollinated with pollen grains suspension concentration at 0.625 g/L plus plus 0.625 g starch/L water (T16).

The obtained fruit weight were (9.84, 10.13, 10.33, 10.36, 10.14, 10.37, 10.61, 10.80, 10.57, 10.71, 10.89, 11.06, 11.52, 11.23, 11.42, and 11.45 g as an average of two studied seasons) due to T1 to T16, respectively.

Such improvement of fruit physical properties i.e. increasing the fruit weight and size might be occurred in response to using diluted pollen grains suspension plus starch concentration for pollination. So, it could be stated that “there is a positive correlation between fruit weight and 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 cause a shortage in the number of fruits per bunch without changing the number of leaves that may induce the 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 easily to identify the fruit set percentage which gave the considerable yield characterized by high fruit quality using either different hand pollination or fruit thinning methods.

B-chemical characteristics

Data in Table (3) indicated that there was an increasing on T.S.S % and Total sugars % with reducing of the pollen grains suspension concentration, and starch concentration in suspension

So, there was a significant increase in T.S.S % and Total sugars due to pollination with 5g/L plus 5 g starch/L water (T1).compared with 0.625 g/L plus 0.625 g starch/L water (T16).

The pollination by diluted pollen grains suspension concentrations at 5 to 0.62 g/L and starch carrier (at

0.625 to 5.0 g/ I water) lead 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.

The reduction of the fruit moisture content is very necessary for improving the quality of such cultivar 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 on lowering the competition that may be occurred between fruits and induce an adequate carbohydrates and other essential foods for the residual ones consequently enhance the fruit maturity and improve its contents of total soluble solids and sugar contents. So, it cold be said that the use of diluted pollen grain suspension has a similar effect like the fruit thinning on improving fruit quality.

These results were supported by the results of (Al-Sabahi et al 2006 and Alabri et al. 2006) who recommended that the use 0.1 g pollen grains/liter of H2O for Helaly Oman date palm. To get an economic yield with good fruit quality. Moreover, El- Salhy et al 2010)and concluded that pollination of Saidy date palm using pollen grain suspension concentration at 2.5 g/L plus 1g ascorbic acid

In regard of the previously mentioned results, it can be recommended that pollination of the saidy date palm using pollen grain suspension concentrations at 1.25 g/L plus 1.25 or 2.5 or 5.0g starch/L water was sufficient to get a high yield with good fruit quality. The advantages of such pollination method is the reduction of Manpower and duration of pollination, both contributing to the reduction of the cost of pollination. Furthermore, it does not require a highly trained labors as with the traditional technique. It ensures the possibility of pollinating a palm at several times in a short period of time. Moreover, allowing the use of mixture of pollens originating from different sources, ensuring good fertilization, and eliminating the risk of accidents occurring as with the old method of climbing a palm several meters high.

CONCLUSION

The objective of this experiment was to examine the effect of some pollination treatments to innovate an untraditional method in date palm pollination which combines both mechanical pollination, fruit thinning and reducing the quantity of pollen grain so we recommend using 1.25 g pollens plus 1.25 to 5.0 g starch/ litre water It can be said that the use of starch in suspense pending action on the stability of pollen and also served as the carrier for the pollen of flowering alnorat glued to fertilization this leads for a harvest good fruits as well as properties provides the amount of pollen and pollination process offering a good treatment of horticultural and economic aspects

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Tables

Table 1: Effect of pollination with pollens (water suspension pollens) with or without starch carrier in fruit set and fruit weight/ bunch (Kg) of Saidy date palm cultivar during 2011 and 2012 seasons.

Treatment		Characteristics.					
		Fruit set%			Fruit weight/ bunch (Kg)		
		2011	2012	Mean	2011	2012	Mean
5 (g / L) pollen	T_0	62.66	58.24	60.45	10.36	9.59	9.98
5 (g / L) pollen + 5g starch	T_1	73.42	67.36	70.39	10.95	10.51	10.73
5 (g / L) pollen + 2.5 g starch	T_2	69.31	63.72	66.52	10.64	10.23	10.44
5 (g / L) pollen + 1.25 g starch	T_3	66.77	60.08	63.43	10.50	9.79	10.15
5 (g / L) pollen + 0.625g starch	T_4	64.53	58.81	61.58	10.31	9.50	9.91
2.5 (g / L) pollen + 5g starch	T_5	69.00	63.40	66.2	10.59	10.21	10.4
2.5 (g / L) pollen + 2.5g starch	T_6	65.43	59.62	62.53	10.38	9.71	10.05
2.5 (g / L) pollen + 1.25g starch	T_7	61.65	55.20	58.43	9.98	9.22	9.60
2.5 (g / L) pollen + 0.625g starch	T_8	57.24	53.30	55.27	9.5	9.00	9.25

Treatment		Characteristics.					
		Fruit set%			Fruit weight/ bunch (Kg)		
		2011	2012	Mean	2011	2012	Mean
1.25 (g / L) pollen + 5g starch	T_9	62.17	56.55	59.36	10.04	9.39	9.72
1.25 (g / L) pollen + 2.5g starch	T_{10}	59.44	53.21	56.33	9.62	9.09	9.36
1.25 (g / L) pollen + 1.25g starch	T_{11}	55.52	51.02	53.27	9.32	8.67	9.00
1.25 (g / L) pollen + 0.625g starch	T_{12}	51.00	47.11	49.06	8.7	8.13	8.42
0.625 (g / L) pollen + 5 starch	T_{13}	51.94	48.34	50.14	8.82	8.31	8.57
0.625 (g / L) pollen + 2.5g starch	T_{14}	47.81	43.10	45.46	8.23	7.59	7.91
0.625 (g / L) pollen + 1.25g starch	T_{15}	43.76	39.22	41.49	7.68	7.00	7.34
0.625 (g / L) pollen + 0.625g starch	T_{16}	41.22	36.30	38.76	7.25	6.50	6.88
L.S.D. 5%		3.61	4.33	3.97	1.46	1.22	1.34

Table 2: Effect of pollination with pollens (water suspension pollens) with or without starch carrier on Fruit weight (g) Fruit length (cm) and Fruit diameter (cm) of Saidy date palm cultivar during 2011 and 2012 seasons

Treatments	Characteristics								
	Fruit weight (g)			Fruit length (cm)			Fruit diameter (cm)		
	2011	2012	Mean	2011	2012	Mean	2011	2012	Mean
T_0	10.41	10.62	10.52	3.68	3.69	3.69	2.25	2.26	2.26
T_1	9.62	10.06	9.84	3.58	3.68	3.63	2.21	2.24	2.23
T_2	9.90	10.35	10.13	3.67	3.70	3.69	2.25	2.26	2.26
T_3	10.14	10.51	10.33	3.68	3.71	3.70	2.26	2.27	2.27
T_4	10.30	10.42	10.36	3.69	3.70	3.70	2.26	2.27	2.27
T_5	9.90	10.38	10.14	3.68	3.70	3.69	2.25	2.26	2.26
T_6	10.23	10.50	10.37	3.70	3.71	3.71	2.26	2.27	2.27
T_7	10.44	10.77	10.61	3.69	3.72	3.71	2.27	2.28	2.28
T_8	10.70	10.89	10.80	3.72	3.76	3.74	2.28	2.28	2.28
T_9	10.41	10.72	10.57	3.69	3.69	3.69	2.27	2.27	2.27
T_{10}	10.61	10.81	10.71	3.73	3.75	3.74	2.27	2.28	2.28
T_{11}	10.82	10.96	10.89	3.75	3.84	3.80	2.28	2.30	2.29
T_{12}	11.00	11.12	11.06	3.85	3.90	3.88	2.30	2.31	2.31
T_{13}	10.95	11.08	11.52	3.85	3.84	3.85	2.30	2.30	2.30
T_{14}	11.10	11.35	11.23	3.88	3.89	3.89	2.31	2.31	2.31
T_{15}	11.32	11.51	11.42	3.93	3.95	3.94	2.34	2.35	2.35
T_{16}	11.34	11.55	11.45	3.93	3.95	3.94	2.34	2.35	2.35
L.S.D. 5%	0.31	0.36	0.34	0.05	0.04	0.05	0.02	0.02	0.02

Table 3: Effect of pollination with pollens (water suspension pollens) with or without starch carrier on T.S.S % Fruit moisture % and Total sugars of Saidy datepalm cultivar during 2011 and 2012 seasons

Treatments	Characteristics								
	T.S.S %			Fruit moisture %			Total sugars		
	2011	2012	Mean	2011	2012	Mean	2011	2012	Mean
T_0	78.00	78.27	78.10	14.33	14.15	14.24	73.24	73.42	73.33
T_1	76.95	77.93	77.44	15.40	15.04	15.22	72.18	73.02	72.60
T_2	77.88	78.12	78.00	14.45	14.27	14.36	73.13	73.35	73.24
T_3	78.00	78.30	78.15	14.45	14.30	14.38	73.24	73.45	73.35
T_4	78.07	78.44	78.26	14.21	14.19	14.20	73.31	73.73	73.52
T_5	77.92	78.14	78.03	15.05	14.21	14.63	73.09	73.37	73.23
T_6	78.11	78.33	78.22	14.24	14.28	14.26	73.34	73.47	73.41
T_7	78.35	78.92	78.64	14.27	14.03	14.15	73.41	74.03	73.72
T_8	78.60	78.97	78.79	14.15	14.00	14.08	73.81	74.12	73.97
T_9	78.19	78.80	78.50	14.21	14.09	14.15	73.26	73.98	73.62
T_{10}	78.53	79.09	78.81	14.16	13.82	13.99	73.66	74.19	73.93
T_{11}	78.90	79.51	79.21	13.8	13.30	13.55	74.09	74.58	74.34
T_{12}	79.50	79.78	79.64	13.30	13.21	13.26	74.57	74.90	74.74
T_{13}	79.36	78.70	79.03	13.45	14.10	13.73	74.51	73.99	74.25
T_{14}	79.84	80.35	80.10	13.18	12.62	12.90	74.81	75.36	75.09
T_{15}	80.23	80.55	80.39	12.70	12.45	12.58	75.18	75,55	75.37
T_{16}	80.40	80.73	80.57	12.56	12.30	12.43	75.41	75.64	75.52
L.S.D. 5%	**1.12**	**1.04**	**1.08**	**1.26**	**1.13**	**1.19**	**0.59**	**0.58**	**0.59**

Improvement of bowl irrigation system under date palm tree (Tunisian Djérid oasis)

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ABSTRACT

The date palm (*Phoenix dactylifera* L.) is the most important tree Crop in Tunisian Djérid oases. Their growth and their yield depend to the Agricultural practices and Climatic condition changements. An assessment of the bowl irrigation system, which is the most common in Djérid oases, was conducted during the years 2010 and 2011. Field experimental is a plot of 52 ha date palm Deglet Noor Variety. The field experiments were carried out for characterizing the soil hydrodynamic properties and monitoring the soil moisture before and after irrigation. Water supplies scheduling has been evaluated through comparison of volumes supplied to the needs of culture. While the Irrigation performances were determined by measurements in situ of the water distribution efficiency and the water distribution uniformity across the orchard. The results reflect that the water supply is still inadequate and that it is not in accordance with the changing needs of the date palm. The application efficiency Ea water irrigation was variable, but it was still acceptable (Ea ≥ 64%). The choice of the appropriate irrigation techniques and the acquisition of better equipment would certainly improve the performance.

Keywords: Date Palm, Soil infiltration, Irrigation, Irrigation Efficiency

INTRODUCTION

Irrigation in the Tunisian oases is characterized by the variability its techniques (basin irrigation, board irrigation, irrigation by pipe, drip irrigation ...). All these irrigation techniques present at the same time advantages and disadvantages. Badly chosen irrigation system can cause economic loss, inefficiency environmental and social fact. Irrigation as practiced in the oases is not conducted according to the real needs of crops. This management method can induce water stress and consequently yield losses, or rather a large water supply and a waste of the resource (Anonym 1, 2010). The present study aims to monitor and assess the technical irrigation by cuvette of date palm orchard in the Eljerid oases (case of oasis Dégueche). Eljerid region belongs to Saharan bioclimatic zone higher costs in winter. This climate is characterized by very high summer temperatures, often by low rainfall (250mm/an) (Anonym, 2003) and irregular, intense radiation, low relative humidity and high evapotranspiration (annual average is about 2139 mm). These climatic factors directly affect the production and quality of dates in particular Deglet Nour variety. The best dates are harvested in areas where the relative humidity of the air is low enough without excessive dryness (Anonym 2, 2003).

MATERIALS AND METHODS

The experimental tests were carried out in a farm area of 1 hectare. This farm is ocated in the Degueche oasis plot which called “EL Manachi”.

The experiments focused on three issues:

- Characterization of soil
- Followed by the irrigation conduct
- Assessment of irrigation performance

1-Experimental site

The tests were conducted in "EL Manachi" oasis which located in Agricultural Training Center of date palm. This oasis covers 52 hectares, it is 50 years old and it has a cropping system in three stages. In addition to palm trees, there are fruit trees and annual crops temporarily during the relatively wet year. The planting density is 150 palms / ha over 85% of "Deglet Nour". But this density increase to 200, even 300 feet / ha with other fruit trees. Thus, the production of "Deglet Nour" is reduced compared to modern oasis where only the date palm is grown. Yields subcultures are relatively good and the conduct of this palm is acceptable.

The technique is widespread irrigation basins with concrete Séguias distribution. The chosen plot covers an area of 1 ha. It is a traditional palm covering 104 date palms variety "Deglet Nour" driving organic. The trees are grown (planted 45 years) and irrigated by wells. Forage crops (especially alfalfa) are often conducted on the first floor.

2- Irrigation Technique plot

Cuvette irrigation technique has been adopted in this oasis plot. Concrete Seguias had been built with dividers and mask modules (Traditional) in order to distribute water between basins. The dimensions of the basin cuvette are variable, but each contains two palm trees which they was spaced 10 m.

3-Analysis of irrigation water

Water samples were also taken to determine the electrical conductivity (EC) of the saturated paste and pH. The dry residue (or the salt concentration) of the irrigation water can be deduced through the relationship:

RS (g / l) = 0.7. EC
Where:
RS: the dry residue of water (g / l)
EC: electrical conductivity (mS / m)
While the pH of the irrigation water was measured using a pH meter.

4-Hydrodynamic characterization of soil

Soil samples were taken every 20 cm from 20 cm up to 120 cm depth in 16 basins. Each sampling point is remote meter date palm. These samples were used to determine the particle size of the soil bulk density, water contents at field capacity and wilting point.

* Particle size analysis

Particle size analysis used to define the soil textural class by determining the proportions of the various components (clay, silt and sand) soil. The proportions of silt and clay are determined by pipetting Robinson Kohn-after dispersion of the colloidal suspension with a reactive dispersant. The sand fractions were determined by dry sieving.

* Bulk Density

The bulk density is the ratio of the dry weight of the bulk volume (volume occupied by the solid + pore volume filled with air and / or the soil solution) from the ground up. The method of determining the apparent density (da) is content to collect soil samples on a specified depth (1.2 m in our case), using identical cylinders of known volume (100 ml).
Therefore determined dry density das then deduce the density (Mkadmi Ch. and Daghari H., 2012):

$$d_{as} = \frac{\rho_{as}}{\rho_e} = \frac{1}{\rho_e} \frac{(p_0 + p_s) - p_0}{V_t}$$

Where:
das: dry bulk density
ρe: Density of water [ML-3]
ρas: dry bulk density [ML-3]
Vt: total volume of the sample [L3]
P: Mass of soil sample costs [M]
Ps: Mass of the soil sample dried at 105 ° C [M]

5- Water contents (TerryL. Prichard)

The volumetric water content of soil (qv) is the ratio of the volume of water retained in the volume of the soil sample. This feature is essential for understanding the behavior of chemical, mechanical and hydrodynamic soil. Several methods are used to determine the soil moisture. Gravimetric method had been used to evaluate soil moisture. Determination of water content is performed by weighing a soil sample before and after drying in an oven at 105 ° C for 24 hours. To calculate moisture content weight we use this relationship (Dhaouadi, 2004):

$$\theta = \frac{(Ph\text{-}Ps)}{(Ps\text{-}PT)} * 100\ \%$$

Where:
θ: water content by weight (%)
Ph: mass of the sample of fresh soil (g)
Ps: mass of dry soil sample (g)
PT: tare mass (g)
Knowing the value of θ, we deduce that:

θv= θ.da

We have two characteristic water content values:
- θcc: water content of the soil at field capacity; this is the

water content at which any deep percolation stops due to hydration abundant. The determination of qcc involves taking soil samples to a depth determined (1.2 m in our case) using identical cylinders of known volume (100 ml). These samples are carefully placed on porous plates soaked in water for 24 hours and then placed in a pressure vessel (3 bars). Finally; we determined the water content by weight difference in weight before and after drying.
θpfp The water content of the soil at permanent wilting point, this is the water content lower limit at which the plant can no longer absorb water from the soil.
Determination of θpfp involves taking soil samples to a depth determined (1.2 m in our case) using a helical auger. These samples are carefully placed on porous plates soaked in water for 24 hours and then placed in a pressure vessel (15 bars).

Finally, we determined the water content by weight difference in weight before and after drying (Dhaouadi, 2004)

6- Infiltration

Infiltration is the process of entering of the water into the ground through its surface (Hillel, 1974). Double ring method was used to perform this test.
The equipment consists of two coaxial rings inserted into the ground to a depth of about 15 cm and supplied with water under a constant load. The outer cylinder (guard ring) ensures a vertical flow in the inner cylinder (Doorenbos J. et Kassam, A.H., 1979). The volume of water infiltrated into the inner cylinder is read directly on the scale.

The following expression used to evaluate K, b and f0 in order to determine KS (Kostiakov, A.N., 1932):

$$I(t) = K.t^b + f_0.t$$

*** The soil hydraulic conductivity at saturation (Ks)**

when t tends to infinity, i(t) tends to a constant equal to f0 often taken equal to the saturated hydraulic conductivity (Ks)(Gerveau E,1998).

$$i(t) = \frac{dI(t)}{dt} = K.b.t^{b-1} + f_0$$

II- Monitoring of irrigation

1- Volume of water supplied

At each irrigation, the average volume of water supplied by Va tree is calculated by:
Va = Qa.Ta

Va = volume (l) with water provided by tree
Qa = average flow at the mouth of the basin (l / min)
Ta = average run time per cup (mn)

To determine the flow rate Q at the entrance of each basin was used a bucket capacity of 12 l and a timer. Each water supply, this manipulation was repeated 16 times, at 16 palms distributed. At each watering, whichever Va corresponds to the average of 16 measurements.

2- Water irrigation input frequencies

From April 15 to May 30, we attended six irrigations conducted on the following dates:
- Two irrigations during the month of April: 19 and 27.
- Four irrigations during the month of May: 5, 13, 23 and 30.

III-Irrigation performance

Irrigation performances are evaluated through the determination of the distribution uniformity (CU), irrigation efficiency (Ec) and application efficiency (Ea) of each water supply.

1- Determination of the distribution uniformity coefficient

To determine the uniformity of the distribution of water across the land, we had to measure the volume Va provided to each of the 16 palm trees.

The uniformity coefficient of distribution (at each irrigation) is then calculated using the following (Southorn N, 1997):

$$CU = 100*\left(1 - \frac{\sum_{i=1}^{i=n} | hi - hm}{(n\ hm)}\right)$$

2- Determination of Ec

Ec is the ratio of the volume of water retained (Vr) in the area provided roots in volume (Va). So every contribution, Va. has been taken equal to the average of 16 measurements at 16 palms previously selected. Vr was determined by taking samples of soil before and after irrigation to determine the moisture of the soil. These samples were taken in the middle (1 - 0.5m) from the trunk of each palm. Each time the sampling was done at a depth of 120 cm with a step of 20 cm. This suggests that the majority of the roots are concentrated in the soil layer (0 - 120 cm) (Southorn N, 1997).

$$E_c = 100.\frac{S.Z_r(\theta_f - \theta_i)}{Q.T_a}$$

Ec = Water application efficiency (%)
S = irrigated area (L2).
Zr = Root depth.

q f = volumetric water content after irrigation (L3 L-3).
q i = volumetric water content before irrigation (L3 L-3).
Q = instantaneous flow rate of the network (L3 T-1)
Ta = irrigation time (T)

3-Determination of Ea

Ea is the ratio of the volume of water retained in Vr water deficit in the zone root. Vr was calculated using the following equation (Southorn N, 1997).

$$E_a = 100.\frac{Z_h(\theta_f - \theta_i)}{Z_r(\theta_c - \theta_i)}$$

Zh = soil humidified depth effectively (L)
Ea = irrigation efficiency (%)
q c = soil water content at field capacity (L3 L-3)

Zh must be higher than Zr

RESULTS AND DISCUSSION

1- Soil Hydrodynamics characteristics

The size chart triangle (triangle texture USA, Appendix 1) was used to determine soil texture of "El Manachie" (Table 1). Taking into account the standard deviations calculated, the obtained texture is coarse. The cultivated soil is a Sandy-silt soil.

2- Bulk density

the bulk density in the experimental zone ranges from 1.48 g / ml to 1.74 g / ml with an average of 1.6 g / ml (Figure 2).

3-Water contents

The soil water content at field capacity (q cc)

The water content at field capacity varies between 11% and 14% depending on depth with an average of 12%, the water content is important to the soil surface and then progressively impoverished in depth. This means that in our soil water retention is more in the superficial zone in the area because there are more profound proportions of silt and clay in the surface area so more amount of water will be retained, compared to deep zone where there is less clay and silt.

The water content at permanent wilting point (q pfp)

The (q pfp) is between 2 and 7 with an average of 5 (Figure 3). After drying the moisture remaining in the soil is unsuitable for the tree, this water is low in the surface area and high beyond 60 cm.

4- Infiltration law

In order to establish the law of infiltration, we used the method of double rings. A regression between sheets of water infiltrated I (t) and the corresponding times is shown in Figure 4. According to the curve of the blade infiltrated water increases with time shows that the water descends rapidly under the action of gravity between the pores of soil.

-The soil hydraulic conductivity at saturation (Ks)

To determine the values of the constants of fit of the equation (14), we have drawn the curve i (t) = f (t) (Figure 5).

According to the curve Ks = 65.82 f0 cm / h.
And expression of the infiltration is as follows:

$$I(t) = 25.94 * t^{0,84} + 10.97 * t$$

Infiltration rate of water is very high because our coarse textured soil bulk density and high moisture down so quickly exceed the root zone.

5- Water irrigation analysis:

The analysis for water irrigation showed that the pH is in the range of 7.82 is slightly alkaline and the electrical conductivity of 1.876 mS / cm which seems very acceptable for date palm cultivation.

6-Irrigation assessment

Water irrigation volumes supplied

The evolution of the volumes was monitored during six irrigations, this shows that the volume of water supplied varies between 29 mm and 39 mm. According to the graph in Figure 6, the volume of water supplied increases progressively from a date to another date irrigation of irrigation. We can explain this increase by increasing of water requirements of date palm.

According to the curve (figure7), we note that the water volume supplied is less than the maximum dose for irrigation 19/04/2011, which explains losing of water of approximately 17 mm. for irrigation 27/05/2011, the water volume supplied is equal to the maximum dose.

For other irrigation, the water volume supplied is more than the maximum dose so the crop water needs are provided without losses.

7-The irrigation performances

- The distribution uniformity coefficient

The spatial distribution of water has been good for 6 irrigations with CU> 90%, which means that the distribution uniformity is excellent and there is no problem in the water distribution.

This homogeneity of water distribution is mainly due to a good maintenance of séguias plot

- The irrigation efficiency Ec

Ec is the key parameter for irrigation because it provides information on the volume of water stored in the root zone can be used to meet the water needs of crops.
For the first irrigation (19/04/2011), Ec was 73%, so we have 70% ≤ Ec ≤ 80%which explains that efficiency is acceptable. For the 27/04/2011 date irrigation, it was 64%, 50% ≤ Ec ≤ 70% which implies that the efficiency is low.

For the irrigation of 5/5/2011, it was 93% so Ec> 90%, which implies that efficiency is excellent. For the irrigation of 13/05/2011, Ec=91%> 90% we note that efficiency is also excellent. The irrigation of 22/05/2011 80% ≤ Ec =83%≤ 90% implying that efficiency is good.for irrigation 30/05/2011 with Ec> 90% implying that efficiency is excellent.

Variations in efficiency between different application dates irrigation varies between 64% and 98% with values of Ec for irrigation and irrigation 19/04/2011 27/04/2011 weak compared to other irrigation which vary between 83% and 98% this weakness is mainly due to the loss of irrigation water percolation and the mismatch between irrigation parameters such as surface, ground slope, permeability and flow.

- Irrigation efficient application Ea

The values of the efficiency of irrigation which provide information on the degree of filling of the tank floor (the root zone) are of the order of 85% for irrigation 19/04/2011 Ea ≤ 80% ≤ 90 % which implies that the irrigation efficiency is good while the other irrigations Ea is around 100% which implies that the efficiency is excellent. Thus Ea of the first irrigation is low compared to other irrigation which this weakness to water loss during penetration with bowls of date palm

GENERAL CONCLUSION

The aim of this present study is the evaluation of the one of many basin irrigation techniques which is the most used by oasis Eljérid farmers: cuvette. The experimental plot covers an area of 1 ha. It is a traditional palm driving organic irrigated basin with variable dimensions each containing two palm trees spaced 10 m, including a network of concrete seguia distribute water between basins.

The irrigation Assessment shows that water supplied increases from one date to another. The analysis of consistency in the irrigation basin shows an excellent uniformity of irrigation for each water supply was estimated by 100%. The irrigation efficiency varies from one to another irrigation this variation may be due to deep percolation. But for the last parameter of the performance evaluation of irrigation is the irrigation efficiency was excellent for almost six irrigations followed.

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Table 1: Texture and soil textural composition of the experimental soil

depth (cm)	Clay (%)	Silt (%) %Lf+%Lg	Sand (%)	Texture (*) (U.S.A.)
0-30	16,2(1,32)	28,0(1,64)	55,9(1,04)	SL
30-60	13,7(0,96)	22,4 (2,04)	64,0(1,37)	SL
60-90	5,0(0,26)	33,7(2,10)	61,4(1,51)	SL

(*) SL: Sandy-silt, S: Sander, LS: loamy Sander, Lf: Limon fine; Lg: Limon gross

Figures

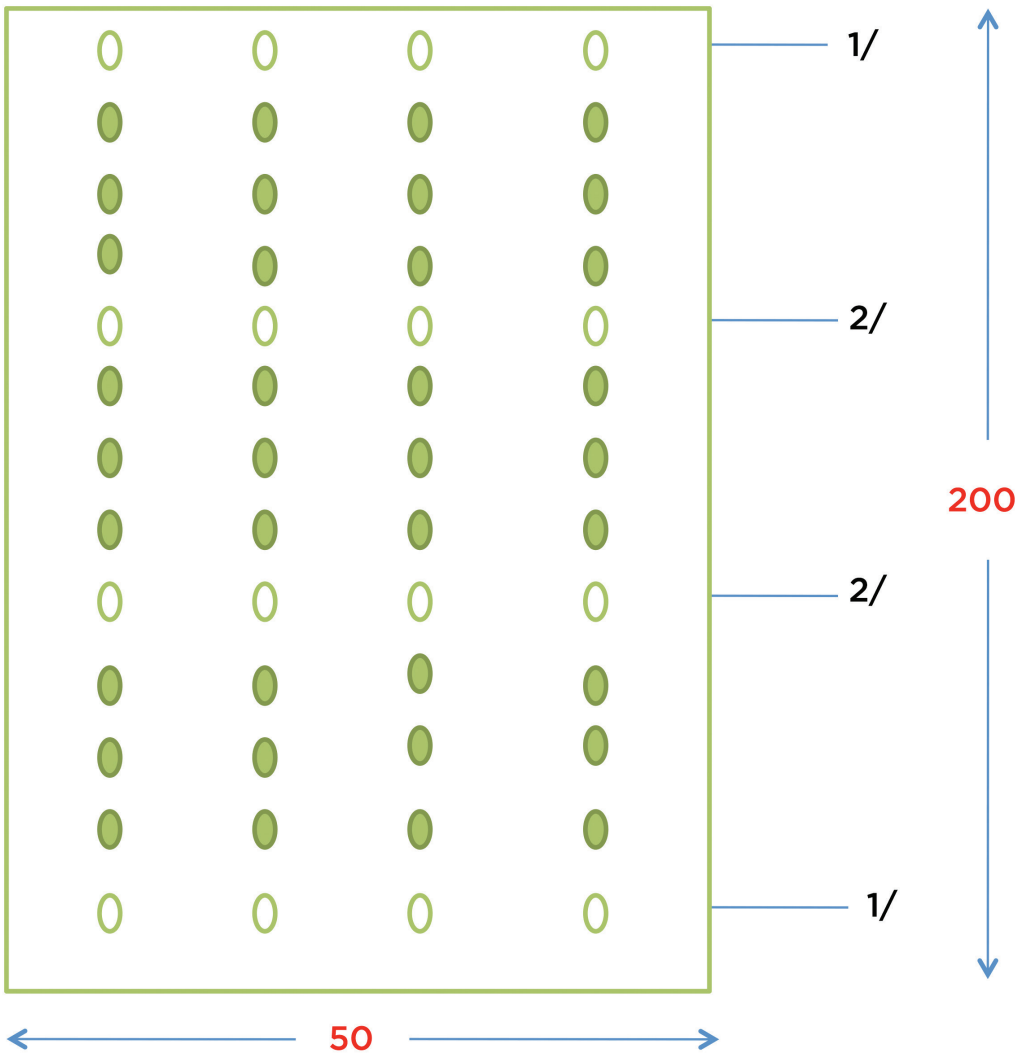

Fig1: A measure of the uniformity of distribution of water in the parcel

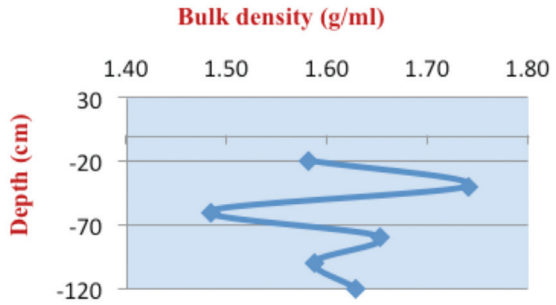

Fig2: Bulk density with depth

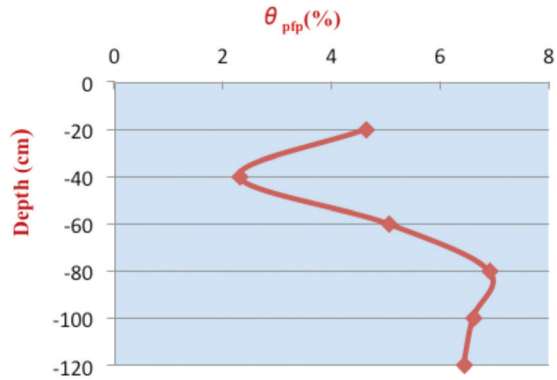

Fig3: Volumetric water content at permanent wilting point with depth

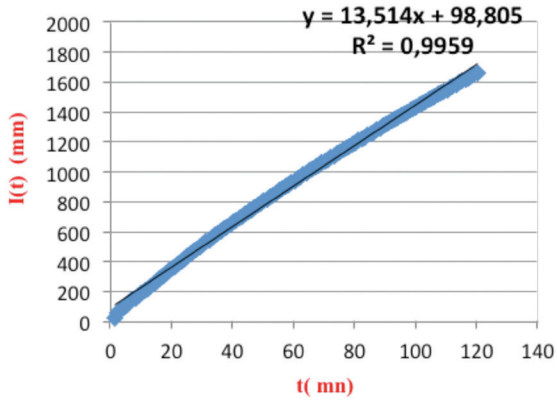

Fig4: the water sheet depending accumulated time

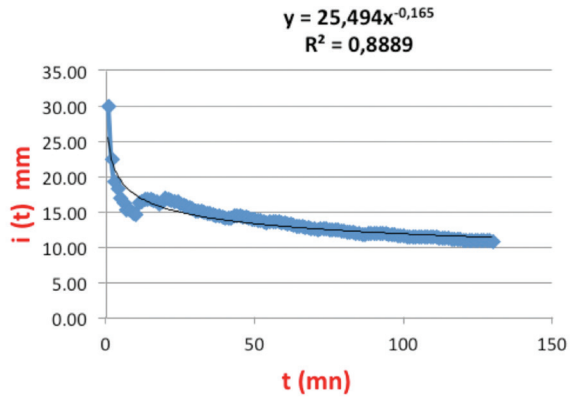

Fig 5: The rate of infiltration according to time

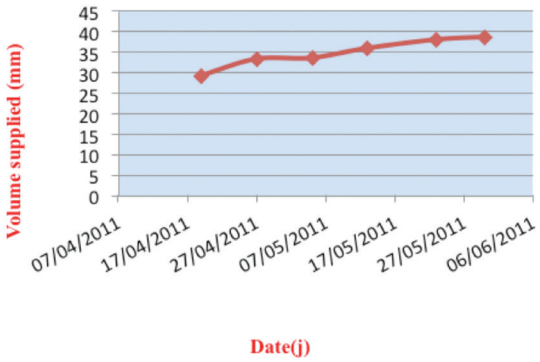

Fig6: Evolution of irrigation water flows during the time

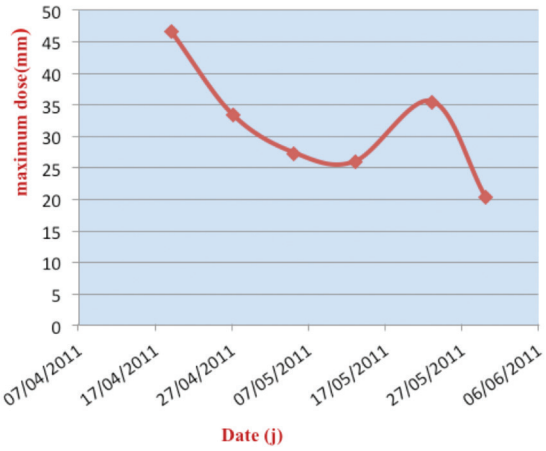

Fig7: the maximum dose provided during the times

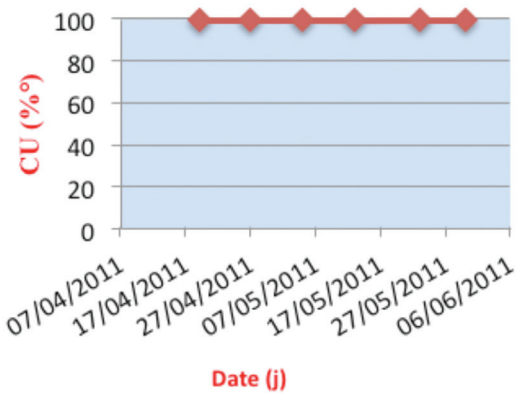

Fig8: distribution uniformity coefficient over time

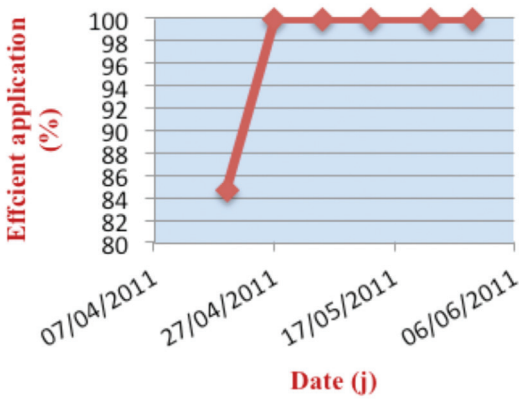

Fig 10: Evolution of Irrigation efficient application over time

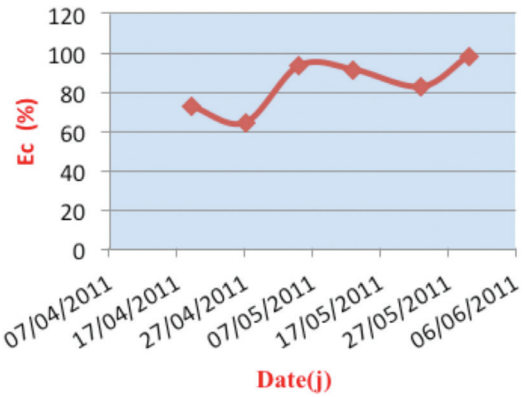

Fig 9: Evolution of the efficiency of water application over time

Review future concerns on irrigation requirements of date palm tree in United Arab Emirates (UAE):call for quick actions

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ABSTRACT

Date palm (*Phoenix dactylifera*) is a very essential traditional tree in a country located in the arid region, like the United Arab Emirates (UAE). This tree is considered as a significant source of food production and plays an crucial role in food security of the country. However, the limitations of natural water resources in conjunction with groundwater depletion and sharp growth in the UAE's population could create critical challenges in providing the irrigation requirements for such economical tree. The main purpose of this work is to investigate whether the future watering requirements of date palm tree could be met and sustained in 2030. This would be done through, reviewing the available irrigation resources and predicting the projected demand (from date palm production and watering requirements), which are required to reach food security in the country. The conclusion indicates that, in the absence of groundwater resources, that may take place in 2030, if all treated domestic wastewater in the UAE "about 578 million m3/year" would be used only to irrigate date palm trees "with irrigation requirements about 640 million m3/year", it would not be enough to cover the watering requirements for palm trees. Consequently, quick actions have to be done from decision makers through irrigation management and strategic planning.

Keywords: date palm, *Phoenix dactylifera*, water scarcity, groundwater depletion, non-conventional water resources, treated domestic wastewater, irrigation requirements, population growth, food security, 2030, United Arab Emirates (UAE).

INTRODUCTION

Throughout history, date palm (*Phoenix dactylifera* L.) has been one of the most essential trees in the arid regions of the world. Since ancient times (3000 BCE), date fruit was one of the most oldest cultivars of fruit crops, which was most probably originated from the olden Mosopotamia area (southern Iraq) or western India (Wrigley, 1995). This valuable fruit, with high nutritional value, played remarkable role in sustaining people's lives in the desert regions (Lambiote, 1982), which generally characterized by harsh environmental conditions and limited natural resources (Zaid and de Wet, 2002). Later on, date cultivation was spread out from its source of origin to the Arabian Peninsula, North Africa and the Middle East (Nixon, 1951). In the eighteenth century, date palm tree has been introduced to new production regions around the globe, including southern Africa, Mexico, Australia, South America and the United States (Chao and Krueger, 2007).

The date palm (*Phoenix dactylifera* L.) is a perennial dioecious monocot, belonging to the family Arecaceae (Al-Hooti, *et al.,* 1997). The name of this tree originated from its fruit; "phoenix" means purple or red "fruit" and "dactylifera" means finger-like appearance of the fruit bunch in the Greek (Sudhersan and Abo El-Nil, 1999). Fully ripe date contains around 67% simple sugars, 25% water

and 8% mainly from cellulose, pectin and vitamins (FAO, 1962). It provides high nutritional value and serves as rich natural energy resource, which makes this crop an ideal cultivar in arid regions, as well as, in any region suitable for their production (Lambiote, 1982). Nowadays, production and utilization of huge quantities of dates are sharply and continuously increasing at commercial global level (Ismail *et al.,* 2006). In reality, while the world population has doubled over the last 50 years, the world production of dates has increased around 3 times, and this trend is expected to continue in increasing (Al-shahib and Marshall, 2003; Chao and Krueger, 2007). Top producers' countries are Egypt, Iran, Saudi Arabia, Sudan and the United Arab Emirates (UAE), Pakistan, Algeria, Iraq and China (Ismail *et al.,* 2006).

In fact, palm trees flower and form fruit when the shade temperature exceeds 18 oC and 25 oC, respectively (Zaid and de Wet, 2002). Although date palm belonging to the xerophyte species, survive under long periods of drought and high temperatures, and adapting to harsh environmental conditions, however, large amounts of watering is required for vegetation growth and high quality production yield (Furr and Armstrong, 1956).

In the UAE, date palm is a very remarkable and precious tree, which has strong religious, traditional and nutritional significance to the local community. It is one of the most important crops in the country, with many great economical and environmental values. Over the last three decades, date production in the UAE has greatly increased; in order to cover the sharp population growth and to reach food security in the region (FAO, 2008). On the other hand, the groundwater reservoirs, which are the main irrigation resource for date palms, have significantly declined (EAD, 2009).

The main objective of this work is to investigate and predict, based on reviewing the literature, whether watering requirements of date palms in the UAE could be met and sustained in 2030, if the current production trend continue in increasing. Besides, this paper will highlight potential recommendations which are crucially required to reach food security in the country, through irrigation management and strategic planning.

The significance of date palm

Truly, date fruit provides natural resource for simple sugar, from glucose and fructose, which is easily absorbed in the human body. Also, date is an extremely rich resource for potassium and contains very low amounts of sodium. Even date seed can be roasted and crushed into powder for different applications, like, date coffee (Aslam *et al.,* 2013). Besides, the trunk of this tree can be used in many applications, such as, as wood and fuel. Furthermore, the leaves can be used to make many products, like bags, baskets, fans, furniture and papers (Chao and Krueger, 2007).

In medicine, date fruit has many potential health benefits which are scientifically proven, related to its tonic effect and antioxidant activities to reduce the damages caused by the free radicals (Aslam *et al.,* 2013). Nowadays, date is greatly under investigation for its possible effectiveness on different illnesses, like diabetes and heart diseases (Ismail *et al.,* 2006).

In the UAE, date palm production is the main crop produced in the country (EAD, 2009), and thus provides great economical value to the farmers and land owners. The maximum date production of each tree is 70 kg, which is purchased by the government at varied prices based on the quality (FAO, 2008a). Different cultivars from date palms grow in UAE, such as, Khalas, Barhee, Fard "Fardh", Ruzeiz "Raziz" and Bumaan (Ismail *et al.,* 2006).

Palm plantation provides diverse significant values in the UAE. It has religious, traditional and social importance to the local society. In the old times, while the life in this region suffers from poor life conditions, date fruit was the rescuer and magical food resource with great nutritional benefits to the local community (Ahmed *et al.,* 1995; El-Behissy *et al.,* 2001). Also, date palms play an essential role in the desert ecological system and provide significant environmental benefits for the indigenous wildlife. Additionally, palms plantation are greatly effective in controlling the desertification and in land reclamation (Chao and Krueger, 2007). For all above mentioned reasons, this precious tree has attracted a great attention locally and globally.

Irrigation resources

The UAE is a young country, with total area around 82,880 km2 and total population estimated to be 9,206 million in 2012 (World Bank, 2012). It is located in the arid region of the world, southern part of the Arabian Peninsula. It opens into two coasts; Gulf of Oman in the east and Arabian Gulf in the west (UAE Yearbook, 2006). The climate is characterized by very high summer temperatures and high humidity rate along the coastal areas reaching 46oC in average and 100% respectively (FAO, 1997). Although, evaporation rates are high, precipitation rates are low and irregular, with average annual rainfall varies from 60 mm to 160 mm (MEW, 2005). Fresh water resources in the county are scarce and limited mainly to groundwater aquifers (Murad *et al.,* 2007).

Based on above mentioned climatic facts, 100% of the watering requirements of the agriculture are depending on irrigation. In the past, all agricultural lands were irrigated using traditional irrigation methods, such as, flood, furrow and aflaj systems (FAO, 2008a). Today, modern irrigation techniques, which were introduced in the mid of 1980s (EAD, 2009), are used (localized, surface and sprinkler irrigation),

which greatly contribute to save around 60% of the irrigation water comparing to the old methods (FAO, 2008a).

Today, there are three major water resources in the UAE, groundwater (4,052 million m3, 70%), desalinated water (950 million m3, 24%) and treated wastewater (319 million m3, 6%), as illustrated in Figure 1 (FAO, 2008a; FAO, 2008b). Comparing to the domestic and industrial sector, the agricultural sector alone consumes about 83% of the total water demand of the country (World Bank, 2013). Over time, the agricultural sector showed huge expansion; from 950 million m3 in 1990 (Murad *et al.,* 2007) to 3,320 million m3 in 2010 (FAO,2013), as represented in Table 1. This was essential to cover the sharp population growth in the UAE, which was extremely increased around 40 folds in just 4 decades, from 231,529 in 1970 to 8,441,537 in 2010 (World Bank, 2012). Besides, the concept of "desert greening" was a great motivator to enlarge the agricultural sector and turn the arid desert into green paradise (EAD, 2009).

Groundwater is the main conventional water resource in the UAE, which is extremely used to cover two sectors; the agricultural and forestry sector (FAO, 2008a). Unfortunately, the high dependency in this valuable resource and the huge consumption rates comparing to recharging ones lead to severe problems, related to saline water intrusion (Al-Zubari, 1998) and the significant depletion in the groundwater levels by 20 to 60 meters, creating real concerns that groundwater would soon dry out and vanish (EAD, 2009).

Consequently, the non conventional water resources have attracted great attention recently in the UAE; in order to cover the huge water demand, including seawater desalination and domestic wastewater treatment (Murad *et al.,* 2007). However, construction and maintenance of desalinization plants are extremely costly (more than US$2 billion). Besides, they have many negative environmental impacts, related to global warming and threat of the marine biodiversity (EAD, 2009). On the other hand, domestic wastewater treated to high treatment standards, up to secondary and tertiary levels, could be reused and recycled safely at cost effective rates, thus act as an attractive sustainable solution to the fresh water scarcity (World Bank, 2011). Nevertheless, since the UAE is one of the most wealthy countries in the world, from oil revenue (UAE Yearbook, 2010), and based on cultural and religious thoughts, treated domestic wastewater is not used in the country for crop production purposes, and used mainly by the forestry sector and for landscaping purposes (Murad *et al.,* 2007; ADSSC, 2007; ADSSC, 2010). Although, there are currently increasing interest to start using this valuable resource for crop production purposes (EAD, 2009).

According to many recent studies, wastewater can be used after adequate treatment in irrigating agricultural crops (Sheikh *et al.,* 1990; Asano and Levine, 1996; Van *et al.,* 2002; York *et al.,* 2008; Pedreroa *et al.,*2010), even it can be used safely for drinking (Tortajada, 2007). Thus, it can be used safely for watering agricultural crops in the country. According to FAO (2010), date palm is among agricultural crops suitable to be irrigated with treated wastewater, and since this tree is the most important economical tree in the UAE, thus it will have a greater priority to be irrigated with the domestic treated wastewater comparing to other agricultural crops.

Irrigation requirements, food security and future concerns

Over the years, date palm plantations and dates production have been dramatically increased in the UAE, from around 8,000 tons in 1970 to over 50,000 tons in 2003 (FAO, 2008b). In 2005, the total area of the cultivated date palm was estimated to be 172,000 ha (EAD, 2009). The main factor that direct this sharp increasing trend is the dramatic population growth in the country, as illustrated in Figure 2 (World Bank, 2012), which consequently requires a parallel growth in dates production as shown in Figure 3 (FAO, 2008b); in order to reach food security. Undoubtedly, dates production sector will continue in increasing the production rates to cover the local and regional market needs (Chao and Krueger, 2007).

Utilized traditional irrigation techniques for date palms in UAE are mainly aflaj and groundwater (FAO, 2008b). Aflaj are a traditional irrigation systems which have high cultural values and had greatly supported date palm oases, however, currently this valued system has almost dried out. Groundwater aquifers have been substantially exploited by private farms. Unfortunately, water withdrawal rates from this valuable resource doesn't have monitoring system and the farmers, who are mainly uneducated people, have severely impacted groundwater levels. Resulted in drying out of 10% of the total wells and causing very high salinity rates, ranges from 3,500 to 23,100 ppm, to 70% of the groundwater aquifers in the UAE (EAD, 2009).

Modern irrigation systems for palm plantations were introduced at research level between the period 1975 and 1984, which include sprinkler, drip and bubbler systems. Costs of these irrigation systems have 50% subsidized by the government; in order to encourage the farmers to replace old techniques by the new and more efficient irrigation methods. Bubbler irrigation is mostly used for palm trees 3 to 4 years old and even used after maturity with a discharge of 360L/hr. This system is highly efficient in using irrigation water with efficiency reaching 80%. Drip irrigation is a localized irrigation system, which releases water slowly and accurately, using drippers that discharge in a range vary from 4L/hr to 24L/hr. It has two systems; online and inline drip systems. This system is greatly used at around 80% in the western regions (FAO, 2008b).

As declared by EAD (2009), the optimum watering requirements for date palm is 14,800 m3/ha. Most of the labor working in irrigating crops, including palm trees, are unskilled and uneducated people. Therefore, real irrigation rates exceeds the optimum rates and enormous amounts of water is discharged, evaporated and lost due to over irrigation practices given to the plantations in a short period of time (FAO, 2008b; EAD, 2009).

As declared by the EAD (2009), agriculture in the UAE is "living on borrowed time", including palm plantations. Groundwater, which is the main irrigation resource, would be vanished within the next 16 to 36 years. The year 2030, could be the first year with no more supply from groundwater aquifers. At the same time, the required supply from groundwater resources, that will be needed to cover palm plantations only, will reach at least 640 million m3 in 2030, as illustrated in Table 2 (estimates based on EAD, 2009). Therefore, if there will be no more supply from groundwater resources, what will be the destiny of the date palm trees in the country?! Which water resource can cope this shortage in watering resources?

At the same time, the population, based on all socio-economic indicators, is expected to at least doubled from 5.8 million in 2007 to be over 12 million in 2030 (World Bank, 2012). Consequently, the required dates production needed to reach food security, as well as, palm trees watering requirements will be at least doubled in 2030 comparing to 2007, as represented in Table 2, if the consumption rates stayed the same as it was in 2007 (estimates on date production based on EAD, 2009).

Similarly, available treated domestic wastewater in the country, which is the most feasible solution comparing to desalination sea water and currently used only for landscaping, will be doubled in 2030 if the life style of 2007 continued (related estimates represented in Table 2 based on FAO, 2013). It worth mentioning that, even if all treated domestic wastewater in 2030, estimated to be 578 million m3/year without any disposal into sea or desert, will be accepted from the public community to be used for irrigating date palm trees, it will not be enough to cover the required palm watering requirements, estimated to be 640 million m3 from groundwater resources, excluding leaching requirements.

Recommended solutions

All the indicators show that palm plantations in the UAE and dates production are expected to continue in a sharp increasing trend. Creating a very challenging situation related to the huge amounts of water needed for the irrigation, with further shortage in the available water resources in the country when the groundwater aquifers will be vanished.

Since, the groundwater aquifers, which is the main irrigation resource, have specific life time expectancy, this problem can't be solved totally, however, it could be best mitigated through different ways including in the first place irrigation management and strategic planning.

Irrigation management could be done through adoption the best agricultural practices and irrigation methods, including deficit irrigation and irrigation scheduling, in order to reduce watering amounts, increase water use efficiency and increase water productivity. Irrigation scheduling for palm trees based on UAE climatic conditions is excellently illustrated in the FAO (2008b), and could be used as an ideal guidance by farmers. Besides, more effort have to be done related to irrigation scheduling through optimization models; to reach the maximum yield with minimal drops. Taking in consideration, the climatologically factors and climate change (EAD, 2009; Schu¨tze *et al.,* 2011(.

The strategic planning and development option could be done through combination of four main strategic options; first, to take positive actions to reduce irrigation requirements (e.g. increase water use efficiency and water productivity). Second, to use the expensive desalinated water (costs $1.75/m3). Third option, is to irrigate with mixture from saline water and brackish water mixed up to acceptable limits. Fourth option, is to use treated domestic wastewater. Another essential strategic option is to develop the planning and development sector, through adoption and implementation of the best practices worldwide in water planning and development, such as, Australian's expertise (EAD, 2009)

CONCLUSION

The future of palm plantations in the UAE is very challenging, in terms of watering requirements. In 2030, the required supply from groundwater resources that will be needed to cover palm plantations will reach at least 640 million m3. At the same time, the groundwater aquifers, which is the main fresh water resource in the country, have 16 to 36 life time expectancy and will soon dry out. Leaving the enormous palm plantations, with irrigation requirements estimated to be 640 million m3, in very critical situation from watering requirements point of view.

Consequently, quick actions have to take place in order to save the future of this precious tree in this country. This can best be done through; first, irrigation management which include adoption of best agricultural practices, irrigation methods (deficit irrigation and irrigation scheduling) and optimization models. Second, strategic planning and development by reducing irrigation requirements, irrigate with mixture from saline water and brackish water and use treated domestic wastewater for irrigation.

Also, treated domestic wastewater application have to be based on priority use, and using the same to irrigate palm plantations; to reach food security, has higher priority comparing to landscaping. Furthermore, public community have to accept the treated domestic wastewater, with availability estimated to be 578 m3 in 2030, as a potential irrigation resource for date palm trees in the UAE. Finally, hard efforts have to be done at farms level; to make the farmers aware about the current facts, related to limited water resources, and to educate them with best irrigation methods and practices, in order to save the future of both the date palm trees and the agricultural sector generally in the UAE.

Finally, it's very clear from this review that, any further expansion in palm trees in the following years and in the agricultural sector generally, have to be under absolute control and have to be cautiously evaluated and managed from decision makers; in order to best fulfill the sustainable approach for the future of agriculture in the country.

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Tables

Table 1: Assessing water demands by agricultural sector (million m3/year) in the UAE.

Year	Agriculture
1990	950
1995	1,300
2000	1,400
2005	3,323[a]
2010	3,320[a]

Source: Murad *et al.,* 2007; except a: FAO, 2013.

Table 2: Summary of important figures in 2007 and estimations for 2030 in the UAE.

Factors	2007	2030
Population (million)	5.8	> 12
Dates Production (tons)	595,000[a]	> 1,190,000
Watering requirements for date palms from groundwater resources (million m3/year)	320[b]	640[b]
Groundwater supply for date palms irrigation (million m3/year)	320[b]	0.0[b]
Total available domestic wastewater (million m3/year)	289	578

a: estimated value for 2005. b: leaching requirements are excluded

Figures

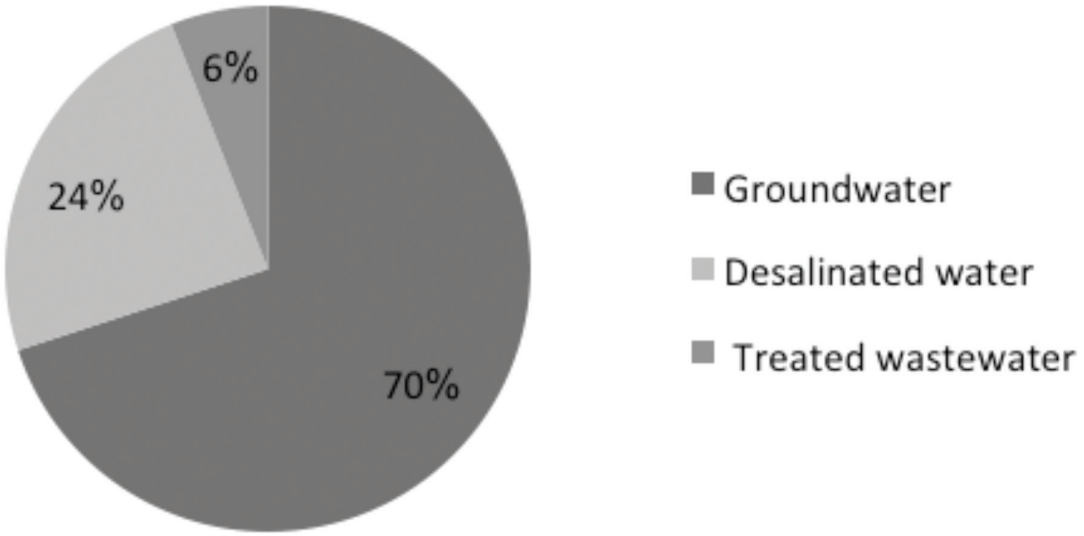

Figure 1: Water resources in UAE (Source: FAO, 2008a).

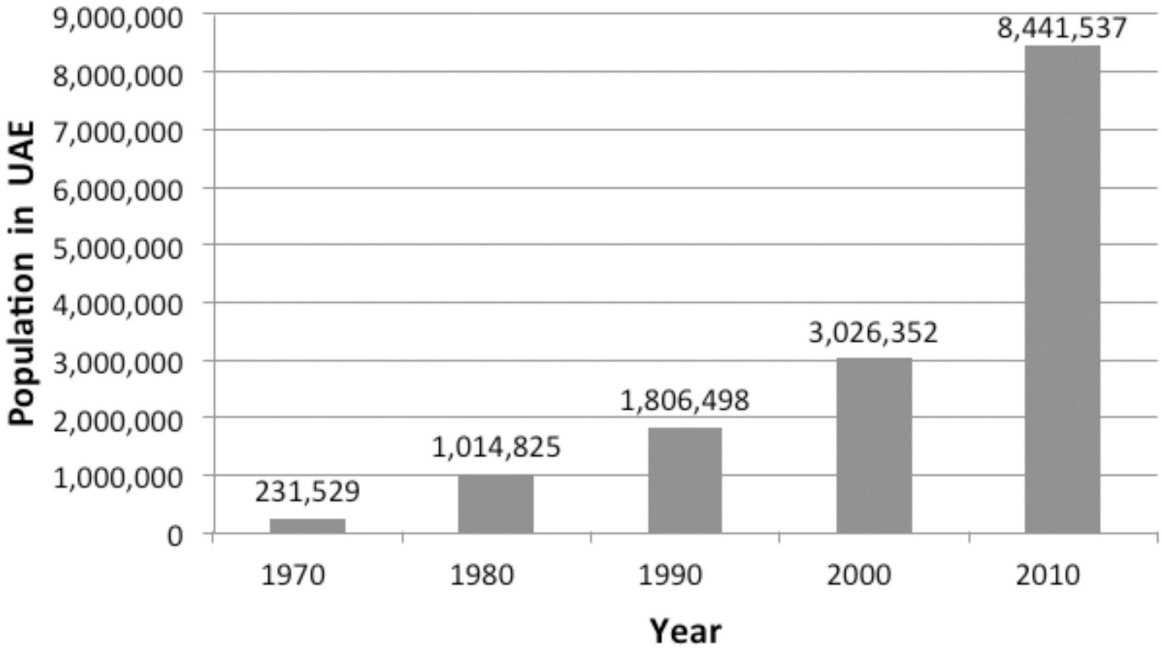

Figure 2: Population growth in UAE (Source: World Bank, 2012).

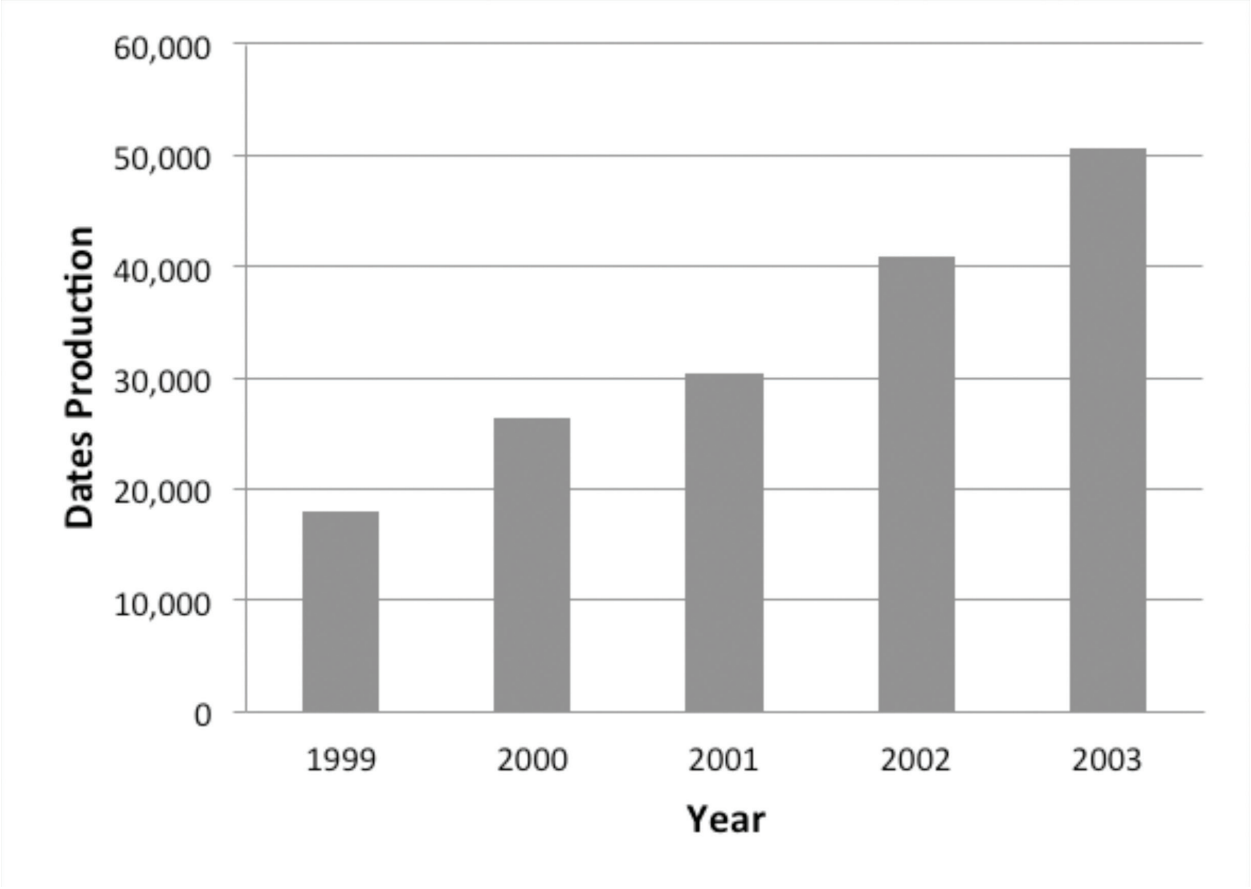

Figure 3: Dates production in the UAE (Source: FAO, 2008b).

The promotive effects of seaweed extract on fruiting of Zaghloul date palms grown under Minia region

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ABSTRACT

This study was initiated during 2011 and 2012 seasons to elucidate the effect of spraying seaweed extract at five concentrations namely 0.0, 0.05, 0.1, 0.2 and 0.4 % on fruiting of Zaghloul date palms. Number of sprays was four.

Carrying out four sprays of seaweed extract at 0.05 to 0.4 % succeeded in improving the leaf area, leaf content of total chlorophylls, percentages of N, P and K, yield and fruit quality in relative to the check treatment. The promotion was in proportional to the increase in concentrations. Meaningless promotion was detected among the higher two concentrations (0.2 and 0.4 %). The best results with regard to yield and fruit quality were obtained with spraying Zaghloul date palms four times with seaweed extract at 0.2 %.

Key words: Zaghloul date palms, seaweed extract, yield and fruit quality.

INTRODUCTION

Poor cropping is considered to be a serious and major problem that faces Zaghloul date palm growers in middle Egypt. Using natural exudates and extracts of biofertilizers namely seaweed extract (extract of *Ascophyllum nodosum*) instead of chemical materials could be the way to improve yield and quality of fruit crops. Previous studies showed that using seaweed extract is favourable in enhancing uptake of nutrients, the resistance of plants to the unfavourable stresses, soil fertility, fruit setting % and activity of microorganisms. It uses as chelated compounds, substitute for organic fertilizers as soil conditioners (Norric *et al.,* 2002 and Aziz *et al.,* 2003). It is a natural source of organic and material fertilizers. It contains more than 60 nutrients and 21 amino acids, natural hormones namely IAA, GA3 and cytokinins and some organic acids (Tung- Yunn *et al.,* 2003).

Previous studies showed that application of seaweed extract was very effective in enhancing growth, nutritional status of the trees, yield and fruit quality of evergreen fruit crops (Gobara, 2004; El- Sawy, 2005; Hegab *et al.,* 2005; Gamal, 2006; Ebeid- Sanaa, 2007; Mouftah, 2007; Ahmed *et al.,* 2008; Hassan- Hoda, 2008; Mohamed *et al.,* 2008; El-Sayed- Esraa, 2010; Mahmoud, 2012 and Mabrouk, 2013).

The target of this study was examining the effect of various concentrations of seaweed extract on fruiting of Zaghloul date palms grown under Minia region.

MATERIALS AND METHODS

This study was carried out 2011 and 2012 seasons in a private orchard situated at Maghagha district, Minia Governorate on thirty 20- years old Zaghloul date palms. Soil texture is silty clay and the palms are planted at 7 × 7 meters apart. The selected palms were irrigated through surface system. Pruning was carried out to maintain leaf bunch ratio at 8: 1 (according to Sayed, 2002). Number of female spathes per each palm was adjusted to ten spathes. Artificial pollination was achieved by inserting five male strands into the female bunch using known high activating pollen source throughout 2 – 3 days after female spathe creaking followed by bagging (Omar, 2007). Each selected palm received the common horticultural practices that are already applied in the orchard except those dealing with using seaweed extract.

This study included the five treatments from five concentrations of seaweed extract namely 0.0, 0.05, 0.1, 0.2 and 0.4 %. Each treatment was replicated three times, two palms per each (30 Zaghloul date palms/ experiment). Randomized complete block design was followed. Seaweed extract (Table 1) was sprayed four times at growth start, just after fruit setting and at one month intervals.

Triton B as a wetting agent was used with all solutions at 0.05 % and the spray was done till runoff (5 L/ palm). The control palms received tap water mixed with Triton B at 0.5 %.

During both seasons, the following parameters were carried out:-

1. Leaf area (m2) (Ahmed and Morsy, 1999).
2. Total chlorophylls (a + b) as (mg/ g-1 F.W) (Moran, 1949 and Wettstein, 1957).
3. Percentages of N, P, K and Mg in the dried leaves according to Piper (1950); Chapman and Pratt (1965) and Wilde *et al.,* (1985).
4. Bunch weight (kg.).
5. Yield/ palm (kg.) at the first week of September.
6. Some physical and chemical characteristics of the fruits namely fruit weight (g.) and dimensions (length and width, cm.) as well as percentages of pulp and seeds, pulp/ seed, total soluble solids %, total and non- reducing sugars % (A.O.A.C., 1995), total acidity % (as g malic acid/ 100 g pulp) according to A.O.A.C., (1995); fibre crude % and total soluble tannins % were determined (A.O.A.C., 1995).

All the obtained data were tabulated and subjected to the proper statistical analysis using new L.S.D at 5 % according to Mead *et al.,* (1993).

RESULTS AND DISCUSSION

1- Leaf area and its content of total chlorophylls and N, P and K:

It is clear from the data in Table (1) that treating Zaghloul date palms four times with seaweed extract at 0.05 to 0.4 % significantly was followed by enhancing the leaf area as well as total chlorophylls and percentages of N, P and K in leaves in relative to the check treatment. The promotion was significantly associated with increasing concentrations of seaweed extract from 0.0 to 0.2 %. Increasing concentrations from 0.2 to 0.4 % failed significantly to promote these parameters. Spraying the palms with seaweed extract at 0.4 % gave the maximum values. Untreated palms produced the lowest values. These results were true during both seasons.

2- Bunch weight and yield per palm:

It is evident from the data in Table (2) that spraying seaweed extract at 0.05 to 0.4 % significantly stimulated bunch weight and yield per palm in relative to the check treatment. There was a gradual promotion on bunch weight and yield per palm with increasing concentrations of seaweed from 0.0 to 0.4 %. Increasing concentrations from 0.2 to 0.4 % had no significant promotion on bunch weight and yield per palm, therefore, from economical point of view, it is suggested to use 0.2 % seaweed extract. Using seaweed extract at 0.2 % four times produced the highest yield from economical point of view. Under such promised treatment, yield per palm reached 188 and 192 kg during 2011 and 2012 seasons. The untreated palms produced 148.8 and 152.0 kg in both seasons, respectively. The percentage of increase due to application of the promised treatment over the check treatment reached 26.3 % during both seasons.

3- Physical and chemical characteristics of the fruits:

It is clear from the data in Tables (2 & 3) that foliar application of seaweed extract at 0.05 to 0.4 % significantly improved fruit quality of Zaghloul date palms in terms of increasing fruit weight and dimensions (length & width); pulp %, pulp/ seed, T.S.S % as well as total and reducing sugars % and decreasing seed %, total acidity %, total soluble tannins and total crude fibre % in relative to the check treatment. The promotion on fruit quality was significantly associated with increasing seaweed extract concentrations. No significant differences were observed on fruit quality among the higher two concentrations of seaweed extract. The best results were obtained due to spraying the palms four times with 0.2 % seaweed extract (since no significant differences were observed among 0.2 and 0.4 concentrations). The untreated palms produced unfavourable effects on fruit quality. These results were true during both seasons.

DISCUSSION

The previous benefits of seaweed extract on growth, nutritional status, yield as well as physical and chemical characteristics of the fruits of Zaghloul date palms might be attributed to the higher own content of essential and on seaweed extract from essential amino acids, minerals, vitamins, organic foods, amino acids and natural plant hormones namely IAA, GA3 and cytokinins (Aziz *et al.,* 2003). It has positive effect on enhancing soil fertility and activity of soil microorganisms (Tung- Yunn *et al.,* 2003). These results are in agreement with those obtained by Gamal (2006); Hassan- Hoda (2008); El- Sayed- Esraa (2010); Mahmoud (2012) and Mabrouk (2013).

CONCLUSION

Treating Zaghloul date palms with seaweed extract four times at 0.2 % gave the best results with regard to yield and fruit quality.

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Tables

Table (1): Analysis of seaweed extracts (According to James, 1994)

Character	Values
Moisture	6 %
O.M.	45 - 60 %
Inorganic matter	45 - 60 %
Protein	6 - 8 %
Carbohydrates	35 - 50 %
Alginic acid	10 - 20 %
Mannitol	4 – 7 %
Total N	1.0 - 1.5 %
Phosphorus	0.02 - 0.05 %
Potassium	10 - 12 %
Calcium	0.2 - 1.5 %
Sulphur	3 - 9 %
Magnesium	0.5 - 0.9 %
Copper	1 - 6 ppm
Iron	50 – 200 ppm
Manganese	5 – 12 ppm
Zinc	10 – 100 ppm
Boron	20 – 100 ppm
Molybdenum	1 – 5 ppm
Cytokinins	0.02 %
IAA	0.03 %
ABA	0.01 %

Table (2): Effect of different concentrations of seaweed extract on leaf area, total chlorophylls & percentages of N, P and K in the leaves, yield, bunch weight and some physical characters of the fruits of Zaghloul date palms during 2011 and 2012 seasons.

Concentrations of seaweed extract	Leaf area (m^2)		Total chlorophylls (mg/ g^{-1} F.W)		Leaf N %		Leaf P %		Leaf K %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 %	1.94	2.01	10.1	10.5	1.82	1.91	0.16	0.18	1.39	1.44
0.05 %	2.01	2.08	10.8	11.2	1.52	1.60	0.21	0.23	1.49	1.52
0.1 %	2.25	2.31	12.0	12.3	1.66	1.74	0.25	0.30	1.60	1.64
0.2 %	2.41	2.50	12.6	13.0	1.79	1.87	0.29	0.35	1.68	1.73
0.4 %	2.45	2.51	12.7	13.1	1.82	1.88	0.30	0.36	1.70	1.74
New L.S.D at 5 %	**0.05**	**0.06**	**0.4**	**0.5**	**0.06**	**0.07**	**0.03**	**0.04**	**0.04**	**0.05**
Character	**Yield/ palm (kg.)**		**Bunch weight (g.)**		**Fruit weight (g.)**		**Fruit length (cm.)**		**Fruit width (cm.)**	
0.0 %	148.8	152.0	18.6	19.0	22.1	22.4	5.50	2.51	2.51	2.53
0.05 %	160.0	162.4	20.0	20.3	24.5	24.8	5.62	2.60	2.60	2.61
0.1 %	172.0	176.0	21.5	22.0	27.0	27.3	5.71	2.69	2.69	2.70
0.2 %	188.0	192.0	23.5	24.0	28.5	28.7	5.81	2.75	2.75	2.77
0.4 %	188.8	193.6	23.6	24.2	28.7	28.9	5.82	2.76	2.76	2.78
New L.S.D at 5 %	**1.4**	**1.5**	**1.1**	**1.2**	**1.1**	**1.2**	**0.07**	**0.06**	**0.05**	**0.04**

Table (3): Effect of different concentrations of seaweed extract on some physical and chemical characteristics of the fruits of Zaghloul date palms during 2011 and 2012 seasons.

Seaweed extract	Pulp %		Seeds %		Pulp/ seed		T.S.S %		Total sugars %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 %	80.0	80.7	20.0	19.3	4.0	4.2	26.0	26.5	19.8	20.1
0.05 %	81.9	82.6	18.1	17.4	4.5	4.7	27.1	27.5	20.8	21.0
0.1 %	83.7	84.5	16.3	15.5	5.1	5.5	28.0	28.4	22.0	22.2
0.2 %	85.0	85.8	15.0	14.2	5.7	6.0	29.2	29.5	23.1	23.3
0.4 %	85.2	86.0	14.8	14.0	5.8	6.1	29.3	29.6	23.2	23.5
New L.S.D at 5 %	**1.1**	**1.0**	**0.9**	**1.0**	**0.3**	**0.4**	**0.7**	**0.6**	**0.5**	**0.4**
Character	**Reducing sugars %**		**Non- reducing sugars %**		**Total acidity %**		**Total soluble Tannins %**		**Total crude fibre %**	
0.0 %	14.0	14.5	5.8	5.6	0.401	0.396	0.69	0.71	0.71	0.70
0.05 %	14.6	15.1	6.2	5.9	0.369	0.360	0.60	0.61	0.51	0.48
0.1 %	15.2	15.8	6.8	6.4	0.330	0.329	0.41	0.38	0.38	0.35
0.2 %	16.0	16.4	7.1	6.9	0.301	0.300	0.30	0.31	0.29	0.20
0.4 %	16.1	16.5	7.1	7.0	0.300	0.299	0.29	0.29	0.28	0.18
New L.S.D at 5 %	**0.4**	**0.3**	**NS**	**NS**	**0.028**	**0.025**	**0.03**	**0.04**	**0.04**	**0.05**

Date Palm Protection

Ecological aspects and pattern of red palm weevil infestation in date palm orchards

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ABSTRACT

The present work was carried out at the Middle East Regional station of red palm weevil, Quassasine, Ismailia governorate during the period of 2006-2008. The obtained results showed that RPW, Rhynchophorus ferrugineus (Oliv.) has two annual broods of activity, the first brood is considered the main and most economic important, this brood was occurred between March and early July. Four overlapping annual field generations were estimated, the first and second generations were the most important. Baited aggregation pheromone traps caught more adult females compared with adult males allover the year. Regarding vertical distribution of RPW infestation on trunk of date palm trees showed that 77.9% of infestation occurred on the lower part of the trunk up till 100cm height. About 98.7% of infestation occurred up till 200cm. Palm trees aged between seven and ten years was the most preferred for infestation. Significant differences were detected between the susceptibility of different varieties to RPW infestation. Such results are very essential for planning integrated management program of red palm weevil.

Key words: *Rhynchophorus ferrugineus*, Ecology, Pheromone traps, Infestation pattern.

INTRODUCTION

The date palm and date fruits are hosts for many insects and diseases which are seriously enough to inflict heavy losses if left uncontrolled. The red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) is considered the most destructive insect pest of date palm trees which invade date palm plantation in the Arab region since 1985. 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 (Ferry and Gommez, 2002). So, nowadays the date palm crop in the Arab countries is under threat. Because of the concealed nature of red palm weevil larvae (The main stage cause the injury), effective methods for control this pest have been difficult to develop. During the last two decades all efforts to control *R. ferrugineus* in the Arab countries, focused on the use of traditional insecticides, modified cultural practices and recently pheromone traps (Abraham, *et al.* 1998). 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).

The present study was aimed to through light on the seasonal abundance and pattern of red palm weevil infestation in date orchards as essential ecological information for construct management program.

MATERIALS AND METHODS

Field Experiments

Field trapping procedures was conducted based on number of adult captured weekly by baited aggregation pheromone traps. The recommended bucket traps

were distributed uniformly in the selected severely infested area at Quasasine district, Ismailia governorate for one complete year (Jan. – Dec. 2007).

Trap Design and Components

Bucket design traps were used in the present study. The traps were inserted slightly in the soil surface; a number of rounded holes were made to allow adult weevils to enter inside the traps safely and easy. The used traps commonly consist of plastic bucket (nine liters in size). The bucket was punctured around its wall with six holes each of 2.5 cm diameter at 15 cm from the bottom; another three holes of the same size were made in the cover. The commercially used pheromone "P028 Ferrolure +, 700 mg Lure" is a synthetic pheromone lures i.e., a mixture of 4-methyl-5-nonanol (nine parts) and 4-methyl-5-nonanone (one part), the purity of both components > 95 % imported from chem.. Tica Natural, Costa Rica was used for the present field trails. Pheromone sac was hanged underside the trap top surface. The pheromone releases its active chemicals through a plastic membran (3-10 mg/day) from 400 and 1500 N/tube, respectively. Selected kairomone was used as a synergist to activate the potent ability of releasing ethyl acetate blooms. Ethyl acetate bottles however were hanged from the underside surface of the trap top releasing chemicals through a fine plastic tube. Pesticide (Bestban 48% EC) was mixed with trap water inside bucket traps to prevent scape of captured weevils.

Monitoring the Fluctuation in the Population Activity of Adults

The changes in the population density of adults were determined by number of captured *Rhynchophorus ferrugineus* adults based on aggregation pheromone traps distributed uniformly in the selected area. Number of collected weevils caught in the pheromone traps was counted weekly, sexed, and grouped into date record contains monthly figures. All traps were maintained weekly. Ethyl acetate Kiromone and pheromone capsules within each trap were changed every 6-11 week according the seasons. The sum of half monthly counts of RPW caught in aggregation pheromone traps allover one complete year were worked out according the formula suggested by Audemard and Millaire, 1975 and Iacob, 1977 to estimate the field annual generations.

Varietal Resistance of Date Palm:

Regular visits were done in the farms of date palm trees during the period of Jan.-Dec., 2007. Weekly inspections of date palm trees were carried out to examine the infestation of the studied varieties (Zaghloul, Hyani, Semmani, Ommahaat, Omry and unknown (seed)). The height of infestation along the trunk, number of infested palm trees, with special concern to the age of infested date palm trees were considered.

RESULTS AND DISCUSSION

Seasonal Abundance and Approximated Number of Annual Field Generations

The number of half monthly count of *Rhynchophorus ferrugineus* adults caught in food baited aggregation pheromone traps is considered the most suitable measure for sampling infestation, this due to the positive corelationship between the population density of RPW adults and infestation in date palm trees. Figure (1) showed that the presence of two annual broods of activity for *Rhynchophorus ferrugineus*. The first brood is considered the main and most economic important. This brood start with a few number of adult weevils on mid January (1 weevil/ 12 traps/ 2 weeks), then the captured weevils increased gradually uptill mid February and rapidly increased on the second half of February, thus forming a broad peak, reaching its maximum during the first week of march (61 weevils/12 traps/ 2 weeks). The population continued high till the beginning of July, this blunt brood of activity may be includes three overlapping field generations. The overlap of generations in case RPW is attributed to the prolongation ovipostion period which extended for about two months and the long cycle of all developmental stages Hussein, *et al.* 1998 and El-Mohanna, *et al.* 2000 which allowed the interference of adult emergence from different generation.

From the beginning of August the population of RPW then decreased gradually reaching the lowest level during the season on the third week of September, (18 weevil/ 12 traps/ 2 weeks). This decline in the population density may be due to the high temperature and dry condition prevailed during July and August. The biological studies demonstrated that temperature between 27 and 30 ºC is the optimum range for development of different stages and flight activity of adults (Hegazy, *et al.* 2001).

The second brood of activity took place from the end of September until the end of November with relatively moderate peak size at the end of October, this peak represent the fourth field generation.

The results obtained in this study are in agreement with the finding of Hagley, 1963 in Coast-Rica who mentioned that population of RPW decreased obviously during the dry season. Weissling, *et al.* 1992 suggested that temperature and humidity may be a key factors governing the flight activity range in California.

The Estimated Number of Annual Field Generations:

A part of the study is dedicated to determine the number of annual field generations of red palm weevil under natural

conditions at Quasasine, Ismailia governorate. This study is based on the fluctuations in the population density of adult weevils caught in pheromone traps. For this purpose the number of weevils were worked out according the methods suggested by Audemard and Milliare, 1975 and Iacob, 1977 as shown in Fig. (2) in which each generation represented by regression line and slope express the developmental rate and economic importance of each generation. The following are briefly description of each generation:

1. First generation

Adult weevils of this generation were appeared from the second week of January and continued up to the second week of March with relatively high population density (first peak) 61 weevils/ 12 traps/ 2 weeks, this generation lasted for about 70 days.

2. Second Generation

This generation took place from the last week of March to the third week of June with similary number of weevils/ 12 traps/ 2 weeks, the peak of this generation occurred at early May and duration for about 90 days.

3. Third Generation

This generation occurred between the last week of June and the third week of September with about 80 days duration and similar population size with the two previous generations.

4. Fourth Generation

Adult weevils of fourth generation occurred in pheromone traps in relatively low number (20 weevils/ 12 traps/ 2 weeks) from the first week of October until the end of the season with peak on first of November. The population density of the fourth generation was relatively low as compared with the previous three generations. Number of weevilsreaches zero in the traps at the end of December as a result of temperature decline.

It could be concluded that both two methods i. e. normal distribution curve and method suggested by Audemard and Milliare, 1975 and Iacob, 1977 namely (Scale gauss) which followed to determine the number of annual field generations of RPW was confirmed each other and demonstrated that *Rhynchophorus ferrugineus* has completed four generations under field conditions. The results obtained are in are in agreement with the finding of many researchers such as Hagley, 1963 in Costa-Rica, Hussein, 1998 in Egypt, Abdel-Latif, 2000 in Egypt and Vidyasagar, et al. 2000 in Saudi Arabia.

Sex Ratio of RPW, *Rhynchophorus ferrugineus* (Oliv.) Caught in Aggregation Pheromone Traps

Data in Table (1) summarizes the total number of the sexed adults of red palm weevil caught in aggregation pheromone traps at four seasons. Data revealed that adult females tend to increase in number in pheromone traps than adult males all over the year especially during winter month. Out of 188 adults caught in winter 118 were females and 70 were males presenting a sex ratio of about 1.68:1. During spring and summer season, sex ratio were quite equal (1.11:1 and 1.12:1 respectively), whereas, out of 334 adults caught during spring, 176 were females and 158 were males and out of 250 caughted durng summer, 132 were females and 118 were males. The sex ratios recorded during autumn were quite equal for females and males (1.02:1).

These results are in harmony with the findings of Oehlschlager, *et al.* 1995 mentioned that twice as many females as male weevils were caughted, Falerio and Chellapan, 1999 noticed that the pheromone trap captures were female- dominated, El-Sebay, 2003a & 2003b stated that female density was higher than male density and constituted 52.8-57.8% of the total population in the field. Rao and sujatha, 2004 mentioned that the male to female ratio was 1.00: 1.44.

Vertical Distribution of RPW Infestation on Trunk of Date Palm Trees

The vertical distribution of RPW infestation was studied on date palm trees aged between 8-16 years and 3 m. height at Quasasine district, Ismailia governorate. Regardless the date palm varieties, all infestation site were divided into five groups according its height from soil surface i.e. (0.50 Cm, 51-100 Cm, 101-200 Cm and above 200 Cm) in addition the infestation occurred at trees crown. Data in Table (2) revealed that about 26% of the infestation took place at the trunk between soil surface up till 50 Cm height and about 51% of the infestation occurred between 51 and 100 Cm height, while about 21% of infestation occurred between 101 and 200 Cm height. Meanwhile no infestations were observed above 200 Cm and infestation rarely occurred at date palm tree crown (only 1.29% of infestations were recorded at the tree crown). The infestation which occurred at tree crown was observed after the deeply removed of the green leaves. These miss applications lead to expose the soft tissues of the trunk and release the volatile odor (kiromone) that attracts weevils for egg laying.

It could be concluded that about 78% of infestation with RPW occurred on the trunk up till 100 Cm height from the soil surface and about 98.7% of infestation took place up till

200Cm height, meanwhile no infestation was observed above 200Cm height and only 1.29% occurred at the tree crown

Frequency of RPW, *Rhynchophorus ferrugineus* (Oliv.) Infestation in Relation to Date Palm Tree Ages:

To determine frequency of RPW infestation in date palm tree of different ages, a survey of the infested trees was carried out in adjacent fields of date palm trees. The infestated date palm trees were divided into four categories according its age i.e. (2-6 years, 7-10 years, 11-14 years and above 15 years), the number of infested trees were assessed in each category. Data illustrated in Fig. (3) showed that 18.18% of the infestation occurred in date palm trees aged beteen 2 and 6 years after offshoot transplanting and about 67% of infestation occurred in the second category of date palm trees aged between 7 and 10 years, while the third category aged between 11 and 14 years harboured only 15.15% of infestation. Meanwhile, no infestations were observed in date palm trees of age above 15 years. It could be concluded that date palm trees of age between 7 and 10 years are the most preferred age for red palm weevil infestation, subsequently all attention must be give to protect the young trees of date palm trees.

These results are in agreement with those of Muralidharan, et al. 2000 who mentioned that the young date palm plants (2-5 years) are more prone to weevil infestation and Longo and Tamburino, 2005 stated that the insect causes severe damage to palm trees and can cause death within eight months, especially in trees aged 5-20 years.

Susceptibility of date palm varieties to infestation with RPW:

To determine the susceptibility of date palm varieties to infestation with RPW, a regular visits to date palm orchard at Quasasine district, were carried out during 2007. A number of 5197 trees were carefully examined and classified into different varieties based on the external morphology. The investigated palm trees were divided into three groups based on the number of trees belonging to each varieties, the first is the common varieties (cultivated in large number such as Zaghloul, Hyani and Semmani), the second is not common cultivated such as, Ommhaat and Omry while the third group include the unknown varieties (seed varieties). The numbers of infested trees within each variety were surveyed and recorded. Data in Table (3) showed that the surveyed number of palm trees belongs to both varieties Ommhaat and Omry were sufficient for evaluation. The unknown (seed) varieties showed highly susceptibility to infestation with RPW (out of 160 examined palm trees), six trees were infested, represent 3.75% infestation. Meanwhile the most common varieties, Zaghloul, Hyani and Semmani, showed different susceptible to RPW infestation. Hyani variety seemed to be the most susceptible to red palm weevil infestation (2.30%) compared to Zaghloul (0.82%) and semmani (1.75%).

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Tables

Table 1. Seasonal fluctuations in sex ratio of RPW, *Rhynchophorus ferrugineus* (Oliv.), Quasasine, Ismailia governorate, 2007.

Season	Sex ratio		Total no. of Weevils	
	♂	♀	♂	♀
Winter	1	1.68	70	118
Spring	1	1.11	158	176
Summer	1	1.12	118	132
Autumn	1	1.02	48	49

Table 2. Vertical distribution of RPW, *Rhynchophorus ferrugineus* (Oliv.), Quasasine, Ismailia governorate, 2007.

Infestation height above soil surface	No. of infested Palm trees	Infestation (%)	Accumulated Infestation (%)
0-50 cm	40	25.97	25.97
51-100 cm	80	50.95	77.92
101-200 cm	32	20.78	98.70
Above 200 cm	00	00	98.70
At crown of palm tree	2	1.29	100
Total	154	-	100

Table 3. Susceptibility of date palm varieties to infestation with RPW, Quasasine, Ismailia governorate, 2007.

	Date palm variety	No. of infested trees	Total number of inspected trees	% infestation
Common varieties	Zaghloul	21	2549	0.82
	Hyani	54	2345	2.30
	Semmani	2	127	1.57
Not common varieties	Ommahaat	2	9	22.2
	Omry	1	7	14.28
Seed varieties	Unknown(Seed)	6	160	3.75
	Total	80	5197	1.54

Figures

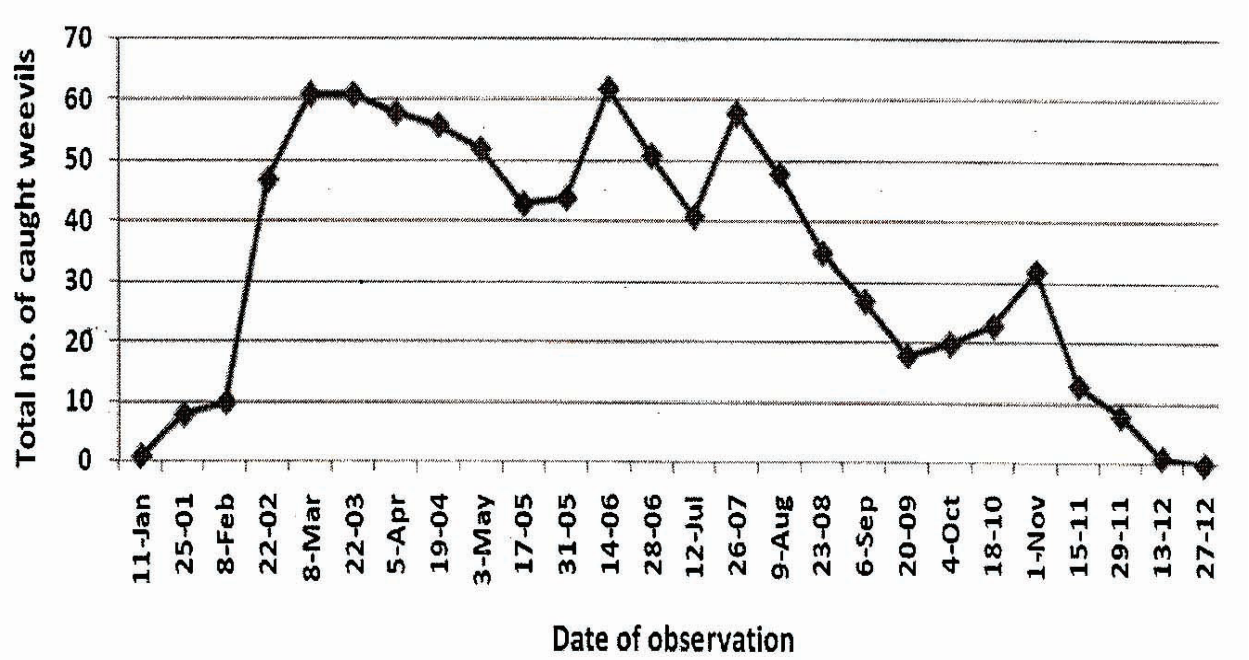

Fig. (1): Fluctuations in the population density of RPW, *Rhynchophorus ferrugineus* (Oliv.) as indicated by total number of weevils caughted in pheromone traps at Quasasine, Ismailia governorate, 2007.

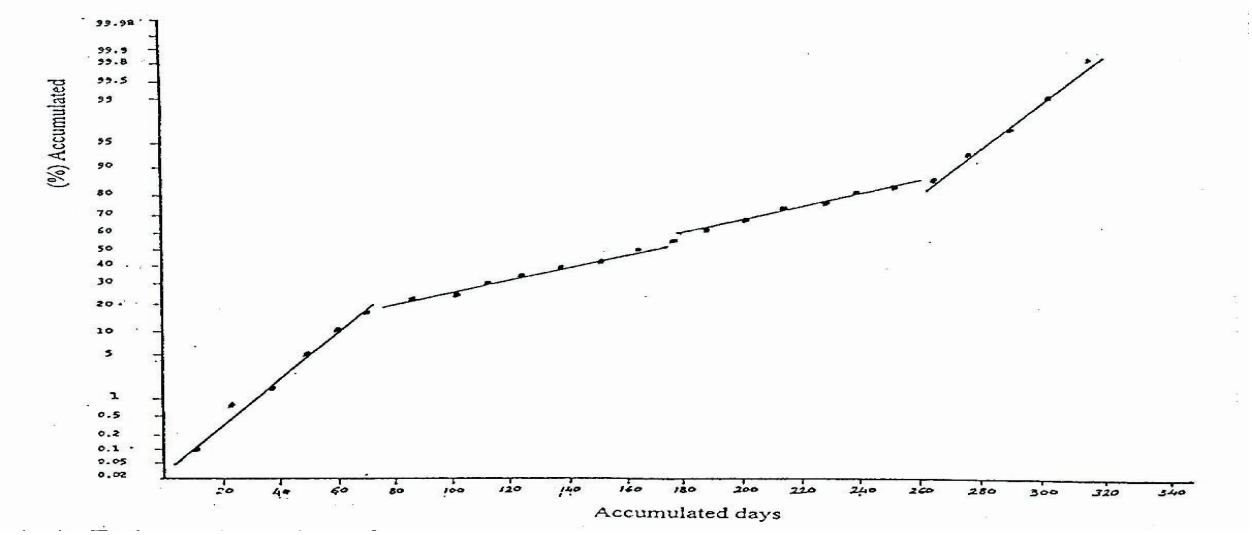

Fig. (2): Estimated number of RPW, *Rhynchophorus ferrugineus* (Oliv.) Aannual field generations, Quasasine, Ismailia governorate, 2007.

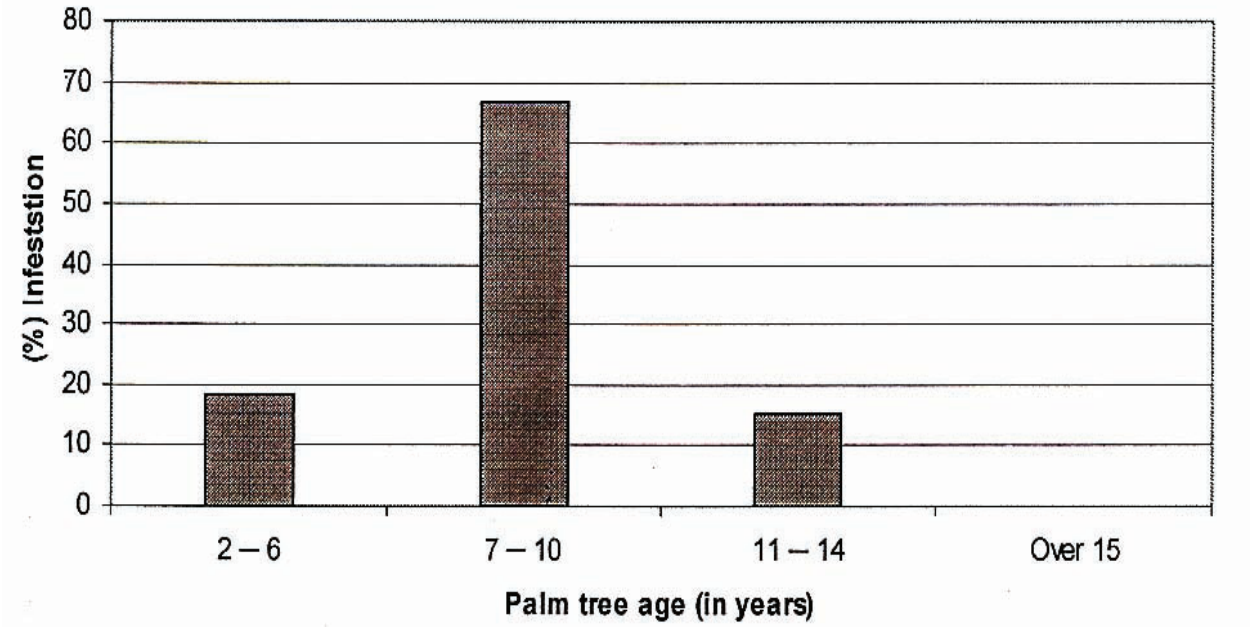

Fig. (3): Percentage of infestation of RPW, *Rhynchophorus ferrugineus* (Oliv.) in relation to date palm age category, Quasasine, Ismailia governorate, 2007.

Factors affecting the efficacy of ethyl acetate in the red palm weevil aggregation pheromone traps

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ABSTRACT

The aggregation pheromone trap is the cornerstone in any Integrated Pest Management Program for the red palm weevil, Rhynchophorus ferrugineus Olivier. Ethyl acetate is one of the important components of this technique; the effectiveness of the ethyl acetate is affected by many factors, such as trap color and date fruit quantity. The results of field trials conducted on date plantations in Al-Rahba (UAE) during May 2005- April 2006, showed that the number of RPW captures in yellow traps were,7.1, 8.4, 8.7 and 9.8 weevils / trap / month when added 150, 250, 300 and 350 g of date palm fruits as a food baits compared by 11.6, 13.8, 14.9 and 15.9 weevils / trap / months for these four treatment with ethyl acetate respectively. The other results showed highly significant differences between the treatments with and without ethyl acetate. Capture rates were 11.8, 20.0, 20.6 and 22.7 weevils / trap / month for white, red, brown and black traps without ethyl acetate respectively compared by 22.3, 34.6, 36.0 and 39.7 weevils / trap / month for these four trap colors with ethyl acetate respectively during January2010-May 2011. It is recommended to use black bucket colored traps containing aggregation pheromone, 350g of date fruits, ethyl acetate and water. The traps should be served, allows and distributed in all date palm plantations all over the year.

Keywords: date fruit quantity, date palm, ethyl acetate, *Rhynchophorus ferrugineus*, pheromone traps, traps color.

INTRODUCTION

The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) is one of the most destructive pests of date palm trees (*Phoenix dactylifera* L.) all over the Gulf Countries (Bokhari and Abuzuhairah, 1992; Gush 1997; Abraham *et al.,*1998; Al-Saoud and Ajlan 2013). Red palm weevil has caused severe damage to date palm trees, in several Middle Eastern countries (Abozuhairah *et al.,* 1996).The insect has caused up to 20% loss of these plantations in Asia and Middle East (Hussein *et al,* 2010). The pest found all over the year (Abraham *et al.,* 1998; Vidyasagar *et al.,* 2000a; Al-Saoud 2007) and the number of female superior numbers on the numbers of male (Abraham *et al.,* 1999; Faleiro and Rangnekar, 2000; Al-Saoud 2009a).

The pest is difficult to control in the early stage of attack because it is an internal tissue borer (Abraham *et al.,* 1998). Initial attempts to control red palm weevil in the Kingdom of Saudi Arabia with insecticides were not successful (Bokhari and Abozuhairah,1992). The Integrated Pest Management (IPM) strategy, modeled on the lines of tackling the pest on coconut in India was implemented in the kingdom of Saudi Arabia, and has successfully suppressed the pest in the date plantations (Abraham *et al.,* 1998). The aggregation pheromone traps has been used successfully monitoring and mass trapping the pest, and is considers the corner stone in any Integrated Pest Programme (Abozuhairah *et al.,* 1996; Perez *et al.,* 1996; Faleiro *et al.,* 1998; Vidyasagar *et al.,* 2000b; Abraham *et al.,* 2000; Al-Saoud, 2011a, Al-Saoud, 2013). The pheromone attracts both male and female weevils (Oehlschlager, 1998; Faleiro, 2000; Abraham *et al.,* 2001; Faleiro *et al.,* 2002; Oehlschlager *et al.,* 2002; Al-Saoud *et al.,* 2010; Al-Saoud and Ajlan, 2013).

The trap effectiveness is affected with many factors, colors (Hallett *et al.,* 1999; Al-Saoud *et al.,* 2010; Al-Saoud, 2013)

trap contents(Al-Saoud, 2009a), food bait, (Nair *et al.,* 2000; Al-Saoud, 2011a) and trap sites (Faleiro, 2005; Al-Saoud, 2011b). The addition of Ethyl acetate (EA) to the aggregation pheromone traps significantly affects on the number of cached weevils and increases the effectiveness of the traps, (Al-Saoud, 2009b; Al-Saoud, 2010; Tigila *et al.,* 1998). The ethyl acetate effectiveness is affected with many factors, trap colors (Al-Saoud, 2013), food bait quantity (Al-Saoud, 2009b) The aim of the study was to evaluate the effect of date fruit quantities and trap colors on the effectiveness of ethyl acetate on red palm weevil aggregation pheromone traps

MATERIALS AND METHODS

1. Study sites

The experiments were conducted in RPW infested date plantations at Al-Rahba, Abu Dhabi (Lat. 24º 28´ N; Long. 54º 22´ E), UAE.

2. Traps and treatments

Pheromone traps were fabricated by using a10-Litre polypropylene bucket with four rectangular (3 x 7cm) windows cut equidistantly below the upper rim of the bucket. The bucket was covered with a lid that had four windows similar to the ones on its sides. The outer surface of the bucket was rough with small projection (1-2 mm) to help the weevils climb to the trap and enter.The upper surface of the lid had a small handle to ease opening the trap and the lower side had a small knob to which a wire was fixed to hold the pheromone and ethyl acetate (EA) dispensers. Each trap contained the following materials: (i) dispenser of the RPW male aggregation pheromone (Ferrolure ™) (4-Methyl-5-Nonanol 90% + 4-Methyl-5-Nonanone 10%) at 95% purity. (ii) 4- 5 Liter of water, with a water level inside the bucket of 2-3 cm below the windows. Water in the traps was replenished so as to keep sufficient moisture. The perforated ladle was used to collect the trapped weevils and to shaken well the traps contents, to prevent growth of any fungi/mould, collection and recorded the weevils captured (male, female), weekly Every trap was shifted to next location after taking weekly results, to avoid location effect on collected insects as recommended by (Faleiro *et al.,* 2002; Al-Saoud, 2006; Al-Saoud, 2010). Water in the traps was replenished so as to keep sufficient moisture.

3. Experimental design and statistical analysis

The experimental design was a randomized complete block design. A distance of 50m was maintained between two treatments (Traps).The data were subjected to analysis of variance (ANOVA) and the means were separated using Least Significant Difference LSD 5% test.

The experimental period, treatments and other trap contents differ according the aim of the each study and were as following:

1-Effect of date frit quantities on the ethyl acetate effectiveens in red palm weevil pheromone traps.

The experiment was done during May 2005 to April 2006, using yellow traps which common used in UAE. The experimental design was a randomized complete block design with four replicates (4 date palm farms) and nine treatments (I- Ethyl acetate +150 g date fruits, ii- Ethyl acetate +250 g date fruits, iii- Ethyl acetate +300 g date fruits, iv- Ethyl acetate + 350 g date fruits, v- 150 g date fruits, vi- 250 g date fruits, vii- 300 g date fruits, viii- 350 g date fruits, ix- Ethyl acetate). The trap were 3-4 m distance from palm trees, they were fixed in hole of 12-15 cm depth in the sand, part of the trap was covered by sand to fix it in safe place, and to avoid trap turning upside-down by wind, animals or any other external factors. Each treatment had dispenser of the RPW male aggregation pheromone (Ferrolure ™) contains 400 mg of active ingredients.

Food bait (dates) was changed once, and the each 20 days. The new pheromone lure was added every 3 weeks, while the new ethyl acetate desponser (Weeil Magent™) containing 40 ml of the active ingredent of EA was added every 45 days during the warmmer months(May – September) and every tow months during the cold period(October- April) to susain the trapping efficiency.

2-Effect of red palm weevil aggregation pheromone trap colors on the effectiveens of ethyl acetate.

The experiment was done during January 2010 to May 2011, with five replicates (5 date palm farms) and eight treatments: (i)-Red colour trap+ Ethyl acetate, (ii)-White colour trap+ Ethyl acetate.(iii)-Black colour trap+ Ethyl acetate.(iv)-Brown colour trap + Ethyl acetate.(v)-Red colour trap without ethyl acetate.(vi)-White colour trap without ethyl acetate. (vii)-Black colour without ethyl acetate.(viii)-Brown colour trap without ethyl acetate, and five replicates (5 date palm farms), where each farm constituted a single replication. All the experimental sites had the above eight treatments and serial numbers were assigned to all traps (1 to 8) at each of the five test farms. Traps were set at ground level, beside the trunk. Each trap contains, (i)- dispenser of the RPW male aggregation pheromone (Ferrolure ™) contains 700 mg of active ingredients. (ii)-350 g date fruits as recommended by (Al-Saoud 2009a) and (iii)-4-5 Liter waters.

Food bait (dates) was changed once a month while the new pheromone lure was added every 45 days during the cold period (October -April) and every month during warmer months (May-September), while the new ethyl acetate desponser (Weeil Magent™) containing 40 ml of the active ingredent of EA was added every month

during the warmmer months(May – September) and every 45 days during the cold period(October- April).

The choice of trap colors in this trial was based on our previous experience where it was seen that superior captures were recorded in red color traps as compared to traps with lighter color shades (Al-Saoud *et al.,* 2010). Hence, we selected red, black and brown colored traps for this study along with the commonly used white (control) color RPW pheromone traps in UAE.

RESULTS

1-Results of study during May 2005 to April 2006:

The weevils were found all over the months of the study in the date palm plantation area in Al-Rahba, Fig. (1). Tthe number of catch weevils/trap/month was differing from month to month. The rate of catches were (5.6, 5.1, 5.4, 5.6, 4.7, 5.3, 13.8, 6.4, 6.7, 14.6, 27.6 and 23.8, weevils/ trap / month), during the period May 2005 to April 2006 respectively. The highest catch (27.6 and 23.8 weevils/ trap) were recorded in March and April, 2006 respectively, and the lowest catch (5.1, 5.4, 4.7 and 5.3, weevils/ trap) were recorded in June, July, September and October 2005 respectively. The numbers of female weevils dominated in the captures in the traps at all months of the study, Fig (1). The sex ratio (Males: Females) ranged between 1: 1.21 in June 2005 to 1: 2.39 in April 2006 with overall mean of 1: 1.95.

The results in Table (1) indicate that there were significant difference between the date fruit quantities in the RPW traps ($F=5.3$, $df=9$, $p<0.005$). The numbers of catches were, 897, 1066, 1130 and 1231 weevils with cached rates of 9.3, 11.1, 11.5 and 12.8 weevils/trap/ month for the traps baited with, 150g, 250 g, 300g and 350 g of date fruits respectively. The mean of RPW captures in the traps baited with 350 g of date fruits dominated over the mean of RPW captures in the traps baited with 150 g date fruits. The percentage increase in weevil captures by the 250, 300 and 350 g date fruit quantities over the 150 g date fruits in the trap were 18.8, 25.9 and 37.2% for these three date fruit quantities respectively, Table(1).

The results in Table (2) show that there were significant differences between the mean of RPW captured in the traps charged with EA and the traps without EA ($F=28$, $df=24$, $p<0.005$). The rate of captures were 7.1, 8.4, 8.7 and 9.8 weevils/ trap/ month, in the traps bitted with 150, 250, 300 and 350 g of date fruits compared by 11.6, 13.8, 14.9 and 15.9 weevils / trap / month for these four treatments charged with ethyl acetate respectively. The percentage increase in weevil captures by the traps charged with EA over the mean of captured weevils in the traps without EA were, 63.4, 64.3, 71.3 and 62.2%, for the traps baited with 150g, 250 g, 300 g and 350 g of date fruit quantities respectively, Table(2).There were no significant difference between the means of captured weevils with the treatments charged with ethyl acetate and, 350 g, 300g and 250 g of food baits, and these three treatments dominated on the traps without ethyl acetate. All treatments dominated on the traps without date fruits (Pheromone and ethyl acetate) which recorded the lowest rate of captures (3.5 weevils / trap / month).

The results in Fig.(2) Show that the RPW pheromone traps charged with EA recorded the highest rates of captured weevils during all the months of the study. The traps charged with EA captured 4324 RPW, with capture rate of 14.1 weevils / trap / month compared by traps without EA, which captured 2693 RPW, with capture rate of 8.5 weevils / trap / month. The percentage of RPW captured were 62.3% and 37.6% for the traps with EA and the traps without EA respectively.

2-Results of study during January 2010 to May 2011:

The results in Fig (3) show that the red palm weevils were found all over the months of the study in the date palm area in Al-Rahba (UAE) during January 2010 to May 2011. The number of catch was differing from month to month. The rate of catches were (11.2, 20.8, 44.6, 47.5, 28.0, 23.2, 21.8, 11.0, 7.2, 8.9, 8.5, 8.7, 19.5, 31.5, 67.9, 57. and 24.4, weevils/ trap / month), during the period January 2010 to May 2011 respectively. The highest catch (67.9 and 57.2 weevils/ trap) were recorded in the months of March and April, 2011 respectively, and the lowest catch (7.2, 8.9, 8.5 and 8.7, weevils/ trap) were recorded in the months of September, October, November and December 2010 respectively. The numbers of female weevils dominated in the captured weevils in the traps at all months of the study, Fig (3).The sex ratio (Males: Females) ranged between 1: 1.8 in June 2010 to 1: 4.6 in October 2010 with overall mean of 1: 2.1.

The results in Table (3) show that there were differences between the mean of catch weevils in different trap colors ($F=87.6$, $df=12$, $p<0.005$). The numbers of cached weevils were 30.8, 28.6, 27.4 and 17.1 weevils/trap/ month for black, brown, red and white traps respectively. The analysis results showed that the all three colors superior as white (control) and the black traps superior on the brown and red traps, no significant differences were found between the mean of cached in brown and red trap. The percentage of collection weevils were 29.6%, 27.6%, 26.4% and 16.4% for black, brown, red and white traps respectively.

The results in (Table 4) show that there were significant differences between the mean of RPW captured in traps charged with EA and the traps without EA, ($F=102$, $df=28$,

p<0.005) . The captures rate were 22.7, 20.6, 20.0 and 11.8 weevils / trap / month for black, brown, red and white traps without EA compared with 38.9, 36.6, 34.8 and 22.3 weevils / trap / month for these 4 trap colors without EA. The black traps charged with EA recorded the highest weevil captures of 38.9 weevils / trap / month, while the white trap without EA recorded the lowest captures of 11.8 weevils / trap / month. The captured rates were 18.8 and 33.2 weevils / trap / month for the traps without EA and the traps with EA respectively.

The results revealed that the total number of catch weevils were 17672 where as male is 5707 and female is 11965 with sex ratio: 1:2.1.

DISCUSSION

The results show that red palm weevil is presented all over the year in date palm plantations in UAE during all years of study, reproduce and increase the infestation severity. Similar results were found by (Abraham *et al.,* 1999; Vidhyasagar *et al.,* 2000b; Al-Saoud and Ajlan, 2013). The peak of activity was in March and April, during all years of study, it may be due to favorable environmental conditions and smell of date palm trees flowers. The lowest activity was in September, October 2005, Fig. (1) and during September, October, November and December 2010,Fig. (3), 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. The overall sex ratio (Males: Females) of the red palm weevil caught in the pheromone traps was 1:1.95 during May 2005 to April 2006 and 1: 1.2 during January 2010 to May 2011, which differs from the results of Abraham *et al*.(1999), who reported a sex ratio 1: 2.68 in favor of females. Al-Saoud (2007), Al-Saoud (2009b) found that the sex ratio of RPW ranged was 1: 1.33 to 1: 2.28. During May 2005to April 2006, and January 2010 to May 2011 periods, the highest red palm weevils were captured during the March and April which is moderate and date palm trees flowering period, in United Arab emirates. The lowest captures occurred during the wormer and colder months, Fig (1 and 3). The EA and date fruits quantity play an important role in the RPW baited pheromone traps, and the captured weevils increase when are the important components (Red palm weevil aggregation pheromone, date fruits, ethyl acetate and water) are combined together.

The results show that the traps charged with EA captured more RPW all over the months of the study, (Fig. 2 and 4). Similar results were found by (Sebay, 2003; Oehlschlager, 2005; Abdullah and Al-Khatr, 2005; Abdullah *et al.,* 2008; Al-Saoud, 2009b; Al-Saoud, 2013). The date fruit quantities and trap colors affect on the effectiveness of EA (Table, 2 and Table 4).The same result was obtained by Abdallah1 and Al-Khatri (2005) in Sultanate of Oman. (Al-Saoud *et al.,* 2010), in the UAE., found that red trap color is more effective than white color to catch the weevil, which recorded the lowest captures, and it was commonly used in UAE. While Kalleshwaraswamy and Jagadish, (2006) results revealed that there were no significant differences in capture rates between red, blue, green, yellow and white traps in India.

Therefore this study reveals that the red palm weevil pheromone traps are those black- colored baited with, red palm weevil aggregation pheromone (contains 700 mg active ingredients), 350 g of fermented date fruits, ethyl acetate and 4-5 L of water captured more weevils during January 2010 to May 2011. These results are similar with Hallett *et al.,* (1999) who recorded the higher weevil captures in black traps compared with the white traps. Furthermore, trap color is known to influence the efficacy of red palm weevil pheromone traps (Kirk, 1984; Hallett *et al.,* 1999; Ajlan and Abdulsalam, 2000; Sansano *et al.,* 2008; Anonymous, 2009; Al-Saoud *et al.,* 2010; Al-Saoud 2013). Ajlan and Abdulsalm (2000) found that the green traps captured more RPW compared with white and yellow traps. Abdallah and Al-Khatri (2005) recorded that the red trap color captures more RPW compared by other trap colors. Sansano *et al.,* (2008) reported that the brown-reddish colored traps recorded the height red palm weevil captures in Spain. Al-Saoud *et al.,* (2010) found that dark- colored traps, in general and red colored ones, recorded more captured weevils compared with white, yellow, pink, orange and blue colored traps in UAE. Al-Saoud (2013) reported that the black traps capture more RPW compared by the brown, red and white traps. These results lead us to use the dark RPW traps colors in particular, black colors contains, 350g of date fruits, 4-5 liter of water, EA and RPW aggregation pheromone in all the highly infested areas in date palm plantation all over the year. Using these traps during the active periods (March to June) in UAE, and use the RPW baited pheromone traps without EA in other areas because the EA is highly volatile, it would increase the cost of the trapping programme. The maintenance of traps is very necessary and replenished the water, change the bait and water monthly and adds new pheromone and EA according the environmental conditions.

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Tables:

Table.1. Effect of date fruit quantities on the number of red palm weevil captures / trap/ month at Al-Rahba(UAE) during May 2005 to April 2006.

Date fruit quantities in RPW trap	No. RPW Captured / 8 traps	Mean ±SE of red palm weevil Captures/trap/ month[1]	Percentage of increase [2]
150 g date fruits	897	9.3±1.3 [b]	---
250 g date fruits	1066	11.1±1.1 [ab]	(18.8)
300 g date fruits	1130	11.5±1.6 [a]	(25.9)
350 g date fruits	1231	12.8±0.9	(37.2)
Mean	1081	11.2±1.1	
LSD 5%		2.0	
F		5.6	

[1]Means with similar letters are not significant different at LSD 5% level.(ANOVA analysis)
[2]Values in parentheses are percentage increase in weevil captures in the traps baited with more than 150 g date fruits over the traps baited with 150 g date fruits.

Table.2. Effect of ethyl acetate and date quantities on the number of red palm weevil captures / trap/ month at Al-Rahba (UAE) during May 2005 to April 2006.

Date fruits quantity in RPW trap	Mean ±SE of red palm weevil captures/ trap[1]		% EA added increase 2
	With EA	Without EA	
150 g date fruits	11.6±1.1[bc]	07.1±1.6[e]	(63.4)
250 g date fruits	13.8±1.4[ab]	08.4.±0.8[de]	(64.3)
300 g date fruits	14.9±2.1[a]	08.7±1.0[de]	(71.3)
350 g date fruits	15.9±1.1[a]	09.8±0.7[ed]	(62.2)
Mean	14.1	08.5	
LSD 5%	2.3		
F	28**		

EA: Ethyl acetate
[1]Means with similar letters are not significant different at LSD 5% level.(ANOVA analysis)
[2]Values in parentheses are percentage increase in weevil captures in the traps charged with EA over the same treatment without EA.

Table 3. Effect of trap colors on the number of red palm weevil captures / trap/ month at Al-Rahba (UAE) during January 2010 to May 2011

RPW trap colors	No. RPW Captured / 10 traps	Mean ±SE of red palm weevil captures /trap[1]	% of increase [2]
Black traps	5286	30.8±3.0 [a]	(82.0)
Brown traps	4832	28.6±2.9 [b]	(66.4)
Red traps	4650	27.4±2.7 [b]	(60.1)

RPW trap colors	No. RPW Captured / 10 traps	Mean ±SE of red palm weevil captures /trap[1]	% of increase [2]
White traps	2904	17.1±2.3 [c]	-------
Mean	4418	26.0±2.7	
LSD 5%		2.0	
F		88.8**	

[1]Means with similar letters are not significant different at LSD 5% level.(ANOVA analysis)
[2]Values in parentheses are percentage increase in weevil captures over white traps.

Table4. Effect of trap colors and ethyl acetate on the number of red palm weevil captures / trap/ month at Al-Rahba(UAE) during January 2010 to May 2011

Trap color	Mean ±SE of red palm weevil captures/ trap[1]		% EA added increase 2
	With EA	Without EA	
Black traps	38.9±3.5 [a]	22.7±2.6 [c]	(74.9)
Brown traps	36.6±3.6 [ab]	20.6±2.1 [c]	(74.8)
Red traps	34.8±3.0 [b]	20.0±2.4 [c]	(73.0)
White traps	22.3±2.7 [c]	11.8±2.1[d]	(89.0)
Mean	33.2	18.8	
LSD 5%	2.7		
F	102**		

EA: Ethyl acetate
[1]Means with similar letters are not significant different at LSD 5% level. (ANOVA analysis)
[2]Values in parentheses are percentage increase in weevil captures in the traps charged with EA over the same traps without EA.

Figures

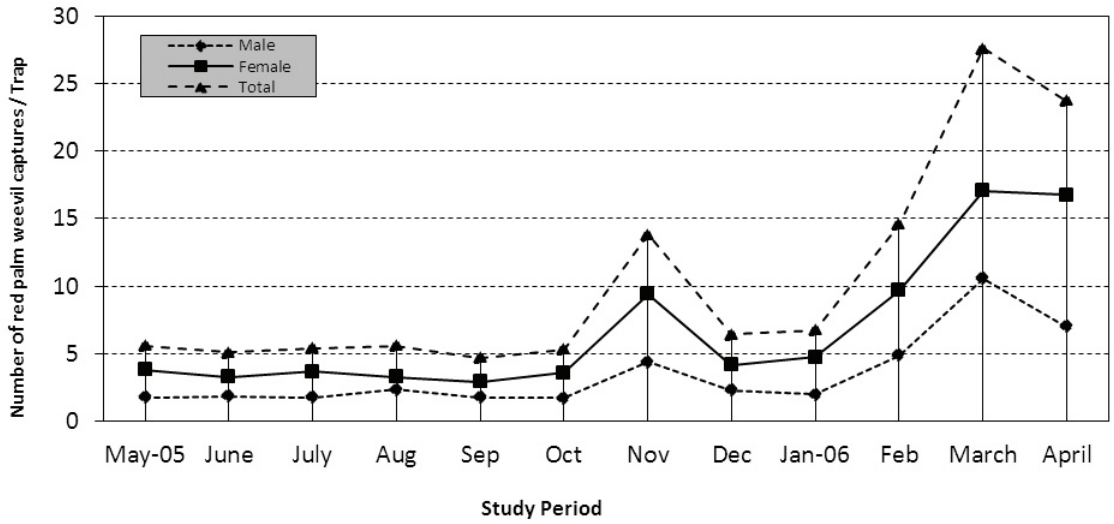

Fig.(1) Activity of red palm weevil, *Rhynchophorus ferrugineus* at Al-Rahba during May 2005 to April 2006

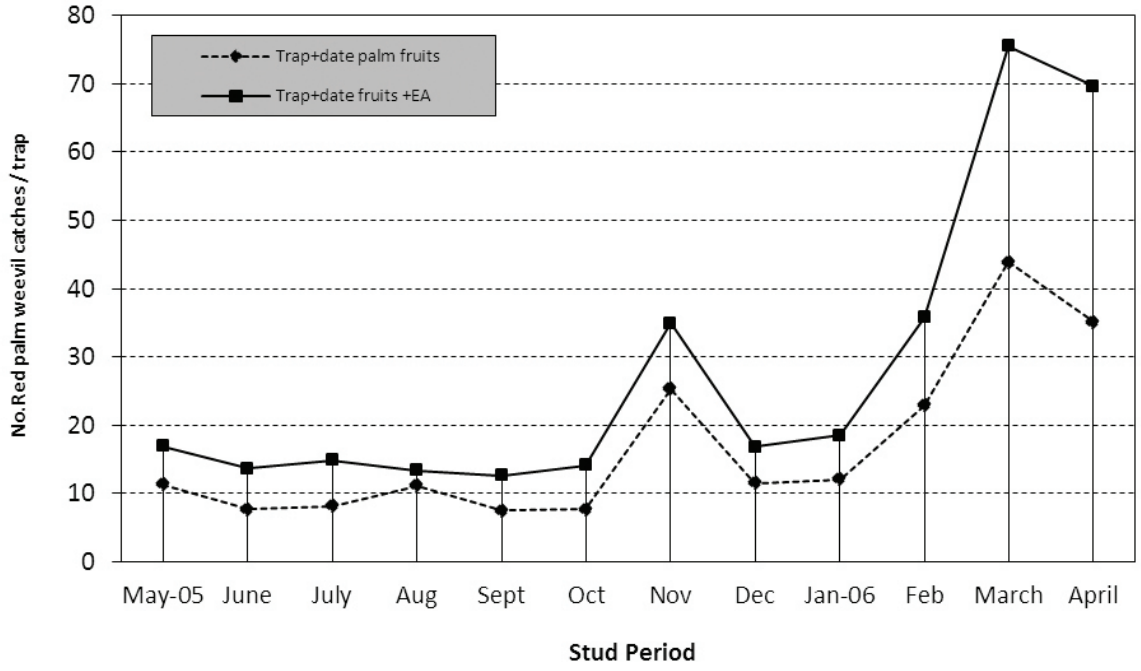

Fig. (2) Effect of ethyl acetate on the number of red palm weevil, *Rhynchophorus ferrugineus* captured in pheromone traps at Al-Rahba during May 2005 to April 2006

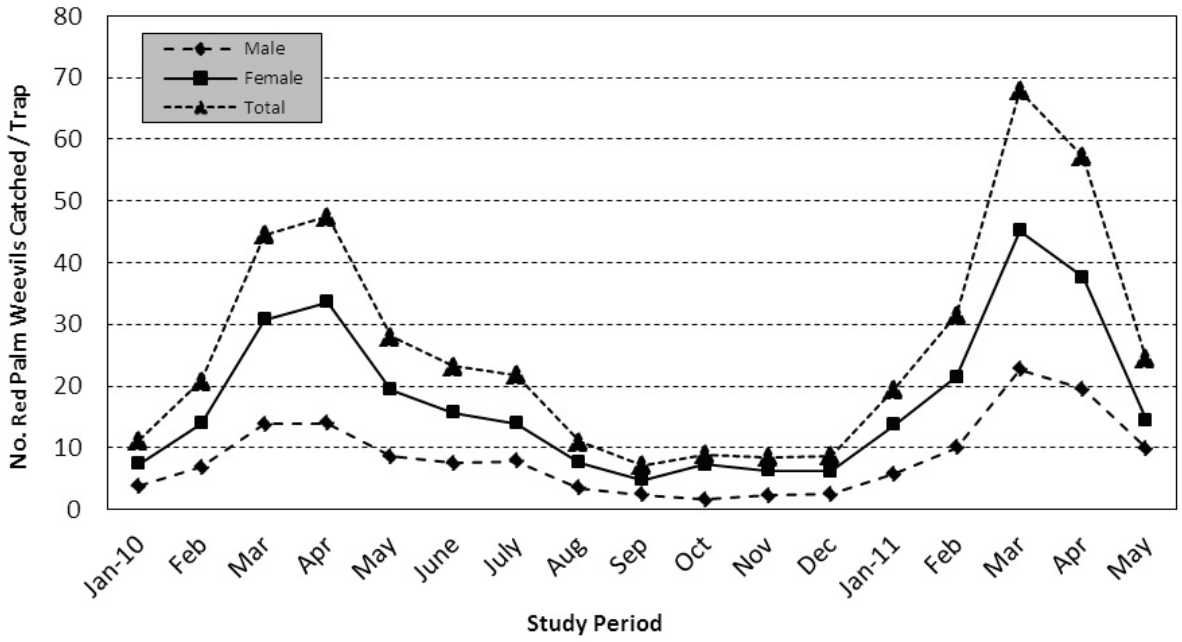

Fig. (3) Activity of red palm weevil, *Rhynchophorus ferrugineus* at Al-Rahba during January 2010 to May 2011.

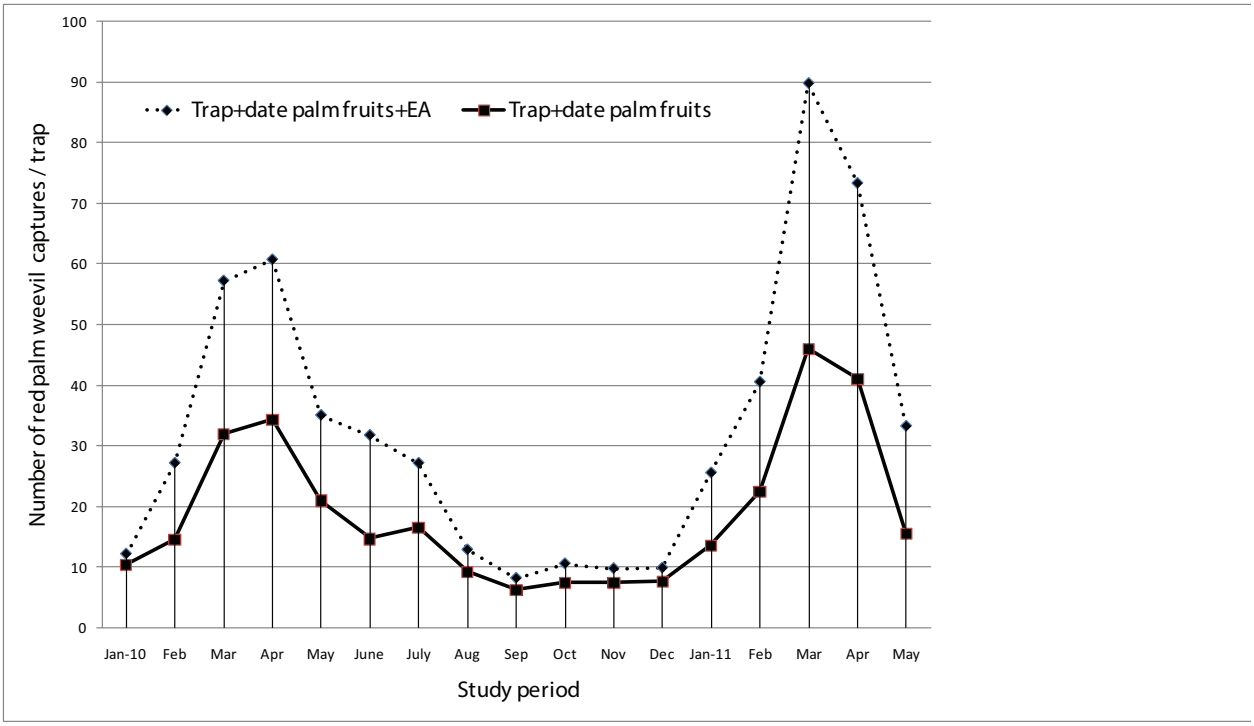

Fig. (4) Effect of ethyl acetate on the number of red palm weevil, *Rhynchophorus ferrugineus* captured in pheromone traps at Al-Rahba during January 2010 to May 2011

Studies on some parasitic and predaceous mites associated with the red palm weevil, *Rhynochophorus Ferrugineus* olivier (coleoptera: curculionidae)

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ABSTRACT

The predaceous and parasitic mites play an important role as biological agents of different pests infesting economic crops. Thirteen predaceous and parasitic mites belong to sub-order Gamasida were recorded associated with the red palm weevil, (RPW), Rhynochophus ferrugineus Olivier (Coleoptera: Curculiodae) in Ismailia Governorate. These mite species; Fascuoropod marginata, Leiodinychus armeri (Uropodidae), Aegyptus rhynchophorus, A. zaheri (Trachyuropodidae), Oodinychus sp. (Trimaturidae), Machrocheles merdarius, Macrocheles sp., (Macrochelidae), Protogamasellus denticus, Proctolaelaps striatus (Ascidae), Sejius paloghi (Sejidae), Cosmolaelaps feeni (Laelapidae), Dendrolaelaps sp., Digamasellus sp. (Digamasellidae) were isolated from adults, pupae (cocoons) and cores around tunnel borded and larvae inside the palm trees. The uropodid and trachyuropodid mites are parasitic on adults and pupae of (RPW), while the other mite species are predators. Biological studies were carried out on the parasitic mite, Aegyptus rhynchophorus when it reared on pupae of RPW and diet of sugarcane under laboratory conditions. Obtained data revealed that both sexes female and male passed through; egg, larva and two nymphal stages before reaching adult stages. Female oviposition period lasted (8.5 & 9.4) days and deposited an average of (37.2 &23.0) eggs with a daily rate of (4.3 & 2.4) eggs when reared on the above mentioned sources, respectively. Female longevity lasted (16.3 & 18.7) days, while male adulthood lasted (13.2 &14.4) days when they fed on (RPW) pupae and diet of sugarcane, respectively.

Key words: Acari; Mites, Red Palm Weevil, *R. ferrugineus*.

INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera : Curculionidae) is an economically importance invasive tissue borer that has been broad host range restricted to palm trees, mostly young trees less than 20 years old, where the stem of the young palm is soft, juicy and easily penetrated (Eppo, 2008), (Salama *et al.,* 2009) and (El-Mergawy and Al-Ajlan, 2011). Biodiversity of mites associated with the red palm weevil *R. ferrugineus* is varying degrees of bio-relationship between each of the associated, ecto, endoparasitic, predaceous, phoretic and fungivorus mites. The parasitic and biocontrol agents of different pests infesting different economic crops. Studies on some mites associated with the red palm weevil have been reported by Gomaa, 2006 who isolated three mite species associated with (RPW). El-Bishlawy and Allam

(2007) recorded new genus and new species, *Aegyptus rhynchophorus* (Trachyuropodidae) associated with pupae and adults of (RPW). Abde-El-Hamed (2009) recorded 14 mite species, associated with differ stages of (RPW) in Egypt. Hassan *et al.,* (2011) studied the biodiversity and seasonal fluctuation of mite families associated with the red palm weevil in Egypt and Al-Dhafar and Al-Qahtani (2012) recorded three mite species associated with (RPW) one of which *Aegyptus alhessa* n. sp. (Gamasida, Trachyuropodidae) as a parasite on eggs, pupae, cocoons and adults of (RPW). Wisniewski *et al.,* (1992) recorded four mite species isolated from *R. ferrugineus* and described. The present study aims to throw lights on some gamasid mites associated with different red palm weevil stages and study the biological developmental stages, fecundity of the parasitic mite, *Aegyptus rhynchophorus* when fed on pupae of (RPW) and diet of sugarcane under laboratory conditions.

MATERIALS AND METHODS

Different stages, larval instars, pupae and adults of RPW were collected from infested palm tree at Ismailia Governortae, Egypt during spring, summer and fall seasons throughout 2009/2010 years. Collected samples of immature stages and adults in addition to materials from their habitats were transferred in plastic boxes (20 x 10 x 10 cm) containing shredded sugarcane stems to the laboratory foe investigation.

Extraction of Mites:

Different stages of RPW were examined individually using dissecting microscope, whereas, detecting mites were removed gently with fine brush or needle from pupae (cocoons), then collected mites were cleared in Nesbitt's solution and mounted in Hoyer's medium for identification.

Identification of mites:

Identification different mite species for their categories, families, genera and species depend mainly on those given by Baker and Wharton (1952), Evans *et al.,* (1961), Lindiquist and Evans (1965), Baker (1968), Hughes (1976), El-Bishlawy and Allam (2007), Eppo (2008) and Abde-El-Hamed (2009).

Source of mite culture:

To obtained the pure culture of parasitic mite, *A. rhynchophorus*, single adult female and male were collected from pupae, then placed in rearing cells and supplied with favorable food and left to lay eggs, which formed the nucleus of its pure culture.

Biological studies:

Eggs of mite, *A. rhynchophorus* were transferred individually to rearing cells (one egg / cell) after hatching to larvae, mites were investigated twice daily and adding suitable of food each of pupae of RPW and pieces of diet sugarcane during developmental stages. Ten replicates were used for each type of food during the biological developmental stages under laboratory conditions of 25+1 ºC and 70 % R.H.

RESULTS AND DISCUSSION

Date palm (*Phoenix dactylifera* L.) is an economically crop, widely cultivated in Egypt and many Arabian countries for its quality of fruit production in addition to numerous known important materials such as fibres, fuel and furniture (FAO, 1984). The red palm weevil (RPW) is considered one of the most economically important tissue boring pest of date palm of the world and it become the major pest of palm in the Mediterranean (Eppo, 2008). The larvae are responsible for damaging the palm and once they have gained access, the death of the palm generally issues. The larva normally never comes to the surface, since; it begins its life inside the palm. The relationship between both predaceous and parasitic mites and different stages of red palm weevil as biocontrol agents are well known to be capable of suppressing its population.

Biodiversity of different mite species associated with the red palm weevil.

Thirteen parasitic and predaceous mites belong to 11 genera; eight families under sub-order Gamasida were isolated from adults and pupae of (RPW) and the cores of around the tunnel bordered by larvae inside the palm trees, Table (1).

1- Family : Trachyuropodidae Berlese

The Trachyuropodid mites were the highest number throughout the course of study. This family represented by two parasitic mite species, *Aegyptus rhynchophorus* (El-Bishlawy and Allam) and *A. zaheri*… …. were found associated with pupae and adults of (RPW) inside the palm trees. Al-Dhafar and Al-Qahtani (2012) recorded and described *Aegyptus alhessa* as a new species and parasitic on eggs, larvae, pupae (cocoon) under elytron of adults.

2- Family: Uropodidae.

The uropodid mites represented by the two parasitic mites: *Fascuropod marginata* and *Leiodinychus karmeri* (G. & R., Canestrini) were found associated with larvae, pupae and adults in high numbers. The parasitoids of both trachyuropodid and uropodid successfully suppressed population density of RPW stages within few days when its found in high numbers, whereas, they killing the different immature stages by sucking their body fluid as well as the larval and pupal weight significantly decrease by increasing numbers of parasitoid mites.

3- Family: Ascidae Voigts and Oudemans.

Ascid mites are known as predaceous mites inhabiting different localities. Two ascid mites; *Prtogamasellus*

denticus and *Proctolaelaps striatus* were recorded associated with larvae and pupae of RPW in moderate numbers

4- Family Macrochelidae Vitzthum.

The two macrochelid mites *Macrocheles maridarus* and *Macrocheles* sp. were found in high numbers during the course of study associated with larvae and pupae, whereas macrochelid mites isolated from core and pupae cocoons. Macrochelids are a wide distribution in different localities and play an important role as biological agents of different pests such as nematodes, housefly (eggs and larvae) and acarid mites.

5- Family : Digmasellidae Evans

This family was represented by two predatory mite species were found associated with different stages of the red palm weevil in rarely numbers.

6- Family: Trematuridae

Oodinychus sp., the only predatory mite species isolated in rarely numbers associated with pupae.

7- Family: Laelapidae Berlese

Laelapid mites represented by only *Cosmolaelaps keni* was found predation on larvae and pupae of RPW I high numbers.

8- Family: Sejidae

This family represented by the predatory mite, *Sejius paloghi* where, it collected from core of palm, pupae and adults in moderate numbers

Biological aspects of the parasitic mite, A. rhynchophorus

The present study was conducted to determine the developmental stages and duration of various life stages, adult longevity and fecundity as well as the effect of food type on biological aspects of the parasitic mite, *A. rhynophorus* fed on pupae of RPW and pieces of sugarcane.

Developmental stages:

Both sexes female and male passed through developmental stages; egg, larva and two nymphal stages before reaching adult stages.

Incubation period

The incubation period of *A. rhynchophorus* lasted (4.1 &5.1) days for female and (4.0 &4.2) days for male when reared on pupae and pieces of sugarcane, respectively.

Larval stage:

Female and male larval stages durated 4.0 days for both when fed on pupae, while when fed on pieces of sugarcane, the period recorded (4.8 and 4.4) days for female and male at the same trend.

Protonymphal stages:

The mean protonymphal period of the parasitic mite female was (4.0 & 4.5) days when fed on the above mentioned types of food, respectively.

Deutonymphal stages:

Female Deutonymphal stage of *A. rhynchophorus* averaged (9.4& 9.8) days when it fed on pupae and sugarcane, while male individuals, the average Deutonymphal stage lasted (7.2 &7.3) days at the same pattern.

Life cycle

The mean duration of life cycle for individuals was (21.4&24.2) and (19.1&19.8) days when the parasitic mite, *A. rhynchophorus* female and male fed on pupae of RPW and pieces of sugarcane at 25+1ºC, respectively.

Adult longevity:

Mean female longevity was (16.3 &18.7) days, when it fed on pupae and sugarcane, respectively. On the other hand, male adulthood lasted (13.2 & 14.4) days at the same trend. The general trend was that obtained durations were significantly longer on pieces of sugarcane than pupae. This applied to both females and males. These results indicated that pupae of (RPW) is the preferred prey. Obtained relative values for males were generally less than females, Table (2).

Female oviposition and fecundity:

As shown in Table (3), female oviposition period and fecundity were significantly affected by different types of food under laboratory conditions of constant temperature and relative humidity. Female oviposition period lasted (8.5 &9.4) days and deposited an average of (37.2 &23.0) eggs with a daily rate of (4.5 &2.4) eggs when reared on pupae and sugarcane, therefore, the pupae as a host of the parasitic mite, *A. rhynchophorus* was attractive for mite survival and development. These results agree with the finding of Sobhi (2006), Abde-El-Hamed (2009), El-Beshlawi and Allam (2007) and El-Dhafar & Al-Qahtani (2012) and Allam *et al.,* (2013).

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Tables

Table (1): The parasitic and predaceous mites associated with different stages of the red palm weevil *Rhynchophorus ferrugineus* on date palm cultivars at Ismailia Governorate.

Families	Species	Remarks
1- Trachyuropodidae	*Aegyptus rhynchophorus*	+++
	A. zaheri	+++
2- Uropodidae	*Fuscuropod marginata*	+++
	Leiodinychus karmeri	+++
3- Ascidae	*Protogamasellus denticus*	++
	Proctolaelaps steriatus	++
4- Macrochelidae	*Macrocheles maridaryus*	+++
	Macrocheles sp.	+++
5- Digmasellidae	*Dendrolaelaps* sp.	+
	Digmamasellus sp.	+
6- Trematuridae	*Oodinychus* sp.	+
7- Laelapidae	*Cosmolaelaps* sp.	+++
8- Sejidae	*Sejius paloghi*	++

High numbers = +++, Moderate numbers = ++, Rarely numbers = +

Table (2): Duration of developmental stages of the parasitic mite, *A. rhynchophorus* when fed on pupae of RPW and pieces of sugarcane at 25+1 ºC.

Parasitic stage	Female			Male		
	Pupae	Sugarcane	L.S.D. at 0.05	Pupae	Sugarcane	L.S.D. at 0.05
Incubation period	4.0+0.2	5.1+0.3	0.796	4.0+0.3	4.2+0.3	0.12
Larva	4.0+0.3	4.8+0.3	0.17	4.0+0.0	4.4+0.2	0.09
Protonymph	4.0+0.2	4.5+0.2	0.16	3.9+0.3	3.9+0.3	0.00
Deutonymph	9.4+1.7	9.8+0.8	0.02	7.2+0.5	7.3+0.8	0.09
Total immatures	17.4+1.8	19.1+1.9	0.33	15.1+0.8	15.6+1.0	0.42
Life cycle	21.4+1.9	24.2+0.8	0.19	19.1+0.8	19.8+1.1	0.41
Longevity	16.3+0.6	18.7+0.5	0.91	13.2+0.6	14.4+0.8	0.94
Life span	37.3+1.5	42.9+1.4	0.97	32.3+1.6	34.2+1.5	0.93

Table (3): Female longevity and fecundity when the parasitic mite, *A. rhynchophorus* fed on pupae of RPW and sugarcane pieces at 25+1 ºC.and 70 % R.H.

Food types	Duration in days			Longevity (days)	Fecundity	
	Pre-oviposition	Oviposition	Post-oviposition		Egg/female	Daily rate
Pupae of RPW	4.1+0.2	8.5+0.4	3.7+0.3	16.3+0.6	37.2+1.5	4.3+0.1
Pieces of sugarcane	4.0+0.3	9.4+0.6	5.3+0.4	18.7+0.5	23.0+1.2	2.4+0.03

Role of the dates volatiles compounds on the date moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) infestation

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ABSTRACT

The study conducted at the INRAA Sidi Mahdi - Touggourt station on the interaction between the dates moth Ectomyelois ceratoniae (Lepidoptera: Pyralidae) and some Algerian date palm varieties, namely Deglet Nour, Ghars and Degla-Beidha reached the following results: For all varieties combined, the rate of infestation by Ectomyelois ceratoniae is about 4%. Among the three varieties studied, the Deglet-Nour is the most infested with a rate of 7.75% and Degla-Beidha is the least infested with a rate not exceeding 1.5%, while Ghars variety, introduced a rate 4.5% of attack. The behavioral tests carried out by means of an olfactometric technical (flight tunnel), we have investigated the response of E. ceratoniae females for the various sources of odors; over 73% of those tested have been flying. 54% of females responded positively to various stimuli, whereas 19.42% of themes have sailed into the flight tunnel, but without choosing a source of odor.In addition, 26.28% of the individuals have shown no reaction to the fumes spread through the air flow sweeping varieties. Depending on the stimuli (positive response), the Deglet-Nour was attractive for 50% of females tested (175 individuals), followed by the Ghars variety with 36% and Degla-Beidha with 14% of individuals tested.

Concerning the volatiles compounds identified by GC. We found that the Deglet-Nour was rich with alcohols (2-propanol, ethanol, 1-propanol and 1-butanol) and the aldehyde (acetaldehyde). On the contrary, the Degla-Beidha was poor for some substances, specially the aromatic part presented by acetaldehyde, where the volatile mix contains only three alcohols included: 2-propanol, ethanol and 1-propanol. On the Ghars variety, in turn, it lacks the aromatic fraction (acetaldehyde), but it is provided with all the alcohol fraction targeted.

Keywords: dates variety, Deglet-Nour, Degla-Beidha, Ghars, flight tunnel, olfactometry.

INTRODUCTION

Date palm currently has an economic importance for Algeria to the extent that it is considered the second source of foreign exchange after oil. The Algerian phoenicicole Heritage is estimated more than 16 million date palm with a production of 492,188 tonnes (Anonyme,, 2012).

Cultivars Deglet Nour, Ghars, Degla - Beidha and Mech-Degla occupy about 70% of phoenicicole heritage. The most productive areas are Oued-Righ, Zibans and Souf (Anonyme, 1996; Anonyme, 1999).

owever, this culture faces several constraints, among other things, the Bayoud, which is an infectious vascular fungus called *Fusarium oxysporum* special form albedinis. In addition, the date moth *Ectomyelois ceratoniae*

Zeller (Lepidoptera: Pyralidae) is considered the most redoubtable pest dates and as the main constraint to the Algerian dates export (Doumandji, 1981).

In Algeria, the economic importance of *Ectomyelois ceratoniae* place it in the second ranks after the Bayoud disease (Doumandji, 1977). According to Munier (1973) *Ectomyelois ceratoniae* may cause damage that can sometimes reach 80 % of the harvest.

Ectomyelois ceratoniae is a polyphagous pest, may ingest multitude of fruits other than the dates. This polyphagia has encouraged more its geographical extension giving it the appearance of cosmopolitanism (Doumandji 1976; Doumandji, 1981). These authors add that, this moth is present in areas that extend from the Hawaiian Islands, Florida, the Caribbean, the northern part of Argentina, the Mediterranean basin (southern Europe, the Middle East and North Africa) the belt from the Sahara desert to Iran, around the Cape in South Africa and Madagascar (Doumandji 1976; Doumandji, 1981).

To better understand this subject, it is considered useful to conduct this study on three cultivars of dates most widespread in Algeria to determine the preferences of the date moth at the oviposition time.

Most of the works done on the interactions *Ectomyelois ceratoniae* - host plants, including the date palm, are focused on the pest - host plant relationships, describing mainly attack strategies developed by the pest, during its life cycle in response the biological needs (nutrition and oviposition) . However, little is known about the factors and behavioral, physiological and / or chemical underlying these interaction mechanisms.

MATERIALS AND METHODS

Two kinds of materials were used for this study . There are three varieties of date palm *Phoenix dactylifera* L. (Deglet-Nour, Ghars and Degla-Beidha) and date moth *Ectomyelois ceratoniae* Zeller (Lepidoptera, Pyralidae).

To get an idea of the action of cultivars on the oviposition behavior of *E. ceratoniae* in the field and in the laboratory, there shall monitor the rate of infestation of selected cultivars. It opted for the method of Warner (1988), which is a weekly sampling of 100 dates for the 20 palm representatives each cultivar. After identification and sexing caterpillars, a mass-rearing is made to have a sufficient biological material. Olfactometric tests under controlled conditions are recommended to study the action of plant odors on the behavior of insects. In this study, the behavioral tests of *E. ceratoniae* are made as proposed by Baker et al. (1991); Cosse et al. (1994) ; Mechaber et al. (2002); Dallaire (2003) and Ingwild et al. (2007). The principle of these tests is to expose fertilized females of the *E. ceratoniae* located in a flight tunnel to airflow sweeping cultivars studied (in pairs) by selecting the following parameters: Flight orientation of moths and their flight duration; number of individuals which touching the odor source or landed at about 10 to 50 cm from the odor source. Each moth touching the odor source or landing at about 10 to 50 cm of this source is considered a positive response against various stimuli.

As to the flight tunnel, is a laboratory device used to measure and compare the behavioral responses of insects under controlled conditions. This tunnel is crafted according to the method described by Cosse et al. (1994). This is a Plexiglass tunnel whose dimension are 180 X 50 X 50 cm. Pure air flow come from an air pump, passes through a plastic hosepipe to a flow meter (rotameter) provides with an active carbon filter to control and purify its flow. It then passes through another micropore filter (0.2 µ) for further purification. This air then reaches the flask filled to 2/3 of its volume with distilled water to moisten it. The air flow is then conducted through two hosepipes to two jars containing the stimuli (dates) to be analyzed. These hosepipes are connected to the flight tunnel.

A comparative study of volatile compounds from three cultivars (varieties) of dates was conducted through a Gas Chromatography (GC), to explore the substances considered by Gothilf (1975); Coss et al. (1994) as oviposition stimulant for the *E. ceratoniae* specie. It is Ethyl Hexanoate, Acetaldehyde, Ethanol, 1- propanol, 2-propanol and 1-butanol.

In this study, statistical analysis methods applied are Factorial Correspondence Analysis (FCA) and Principal Component Analysis (PCA) using Gostat software.

RESULTS AND DISCUSSION

The results show a variation in infestation levels depending cultivars (varieties) . Among the three cultivars studied, Deglet-Nour is the most infested, with a maximum rate of 7.75 %, followed by Ghars (4.5 %) and Degla-Beidha (1.5 %) (Fig.1).
Behavioral testing via the olfactory technique (flight tunnel) have reviewing behavioral responses of mated females in position according to choose between two different odors combinaisons. 73, 42% of the individuals tested took flight, whose 54% responded positively to various stimuli, while 19.42% took the flight but no choice. In addition, 26.28 % of the individuals tested showed no reaction to the airflow sweeping three cultivars of dates. (Fig. 2).

Inactive females can be divided into two groups. There are those who have not responded to pure air (49 individuals), that to say 14%. There are also those who

remained inactive even though they are exposed to various stimuli (43 individuals) whether 12.28% (Tab.1) .

It is noticed that the activity of females is important when exposed to air flow from two stimuli (cultivars) compared to those from a single cultivar combined with pure air.

Moreover, it is noticed that in any combination, Deglet-Nour is the most attractive cultivar. Among the 350 females tested, approximately 50% are attracted by Deglet-Nour, 36% by Ghars and 14% by Degla-Beidha (Fig. 3) . Among the 350 females tested, 188 individuals (54%) responded positively to the air flow from the three cultivars of date. 59% of active females have reached the odor source, while 41% were landed at a distance of 10 to 50 cm from the source, the majority of females have completed their flight in an interval of 1-10 minutes.

The analyzes results of volatiles compounds of the three dates varieties have been detected 5 volatiles compounds among the 6 sought. The cultivar Deglet-Nour is richer in volatile compounds emissions include alcohols (2-propanol, Ethanol, 1 -propanol and 1- butanol) and Aldehyde (Acetaldehyde). In the other side, Degla - Beidha proves poor in aromatic compounds and the volatile bunch includes only 2 - propanol, 1 - propanol and Ethanol . Regarding Ghars cultivar, it is devoid of the aromatic fraction (Acetaldehyde), but it is rich with the entire target alcoholic fraction.

The Factorial Correspondence Analysis (FCA) shows that of the 07 characters analyzed, 3 are discriminating, namely: the infestation rate (INFES), presence or absence of Acetaldehyde (ACTAL) and 1- butanol (1BUTA) . From the data shown in Table 5, it is found that the axis 1 which contains most of the information is explained mainly by the following characters: Acetaldehyde (ACTAL) and 1 -Butanol (1BUTA) who contributed to the inertia explained by axis 1 with 16.1% and 20.1 %, respectively. On the entire graphic, Acetaldehyde (ACTAL) and 1 -Butanol (1BUTA) contributed to the total inertia shown by weight of 13.25 and 10.60% (Table 5).

The cultivars studied are classified into three groups (Fig. 4) :

Group 1: represented by Deglet-Nour, characterized by a high rate of infestation (7.75 %) and the presence of volatile compounds : Acetaldehyde (ACTAL) and 1 -Butanol (1BUTA).

Group 2: represented by Degla-Beidha, characterized by a low rate of infection (1.5%) and the absence of volatile compounds: Acetaldehyde (ACTAL) and 1 -butanol. (1BUTA).

Group 3: represented by Ghars, characterized by means infestation rate (4.5) and by the presence of volatile compounds : 1 -Butanol (1BUTA) and the absence of Acetaldehyde (ACTAL).

The most contributing factors to infection are Acetaldehyde (ACTAL) and
1 - Butanol (1BUTA) . Indeed, Deglet-Nour is more attacked (7.75 %) in that it contains two compounds that are oviposition stimulants for the *E.ceratoniae*.

Degla-Beidha recorded the lowest rate of infection (1.5%), this is probably due to the absence of Acetaldehyde (ACTAL) and 1 - Butanol (1BUTA).

Concerning Ghars cultivar, it presented a single compound, (1- Butanol) . This may explain its intermediate infestation rates between the Deglet-Nour and Degla-Beidha .

The infestation rate in the field and behavioral tests in the flight tunnel are perfectly consistent in varietal choice of this pest. Indeed, RENWICK and CHEW (1994) considered that in Lepidoptera, research, guidance and recognition are the first phase of selecting a suitable site for oviposition . Reactions orientations of females of this moth in flight tunnel reflect a fairly clear difference of the stimulants spectrum emitted by dates of each cultivar. The orientation of the females at the time of oviposition seems to be related to secondary metabolites emitted by ripe dates of each cultivar, which finally determines the rate of infestation.

From the results, it is found that the varietal selectivity of *E.ceratoniae* is apparently related to the composition of the volatile bunch and more particularly the amount of Acetaldehyde and 1-Butanol emitted by each variety.

CONCLUSION

The results of the study on the interaction between the date moth *Ectomyelois ceratoniae* and three cultivars of Algerian dates namely Deglet-Nour, Degla-Beidha and Ghars. This study allowed to retaining the following: The infestation rate in field and olfactometry laboratory tests are in complete concordance. Deglet-Nour is more infested in the field and the most attractive in the flight tunnel. The Ghars cultivar occupies 2nd rank and Degla-Beidha took the 3rd place.

Varietal selectivity of *E.ceratoniae* is apparently related to the composition of the volatile bunch emitted by ripe dates of each cultivar. It may be that the Acetaldehyde and 1 -butanol fraction as olfactory stimulant, is critical to the *E.ceratoniae* females oriententation at the oviposition moment for choosing a laying eggs site.

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Table

Table 1. Effectifs et comportement des individus d'*E. ceratoniae* exposés aux différentes odeurs dans le tunnel de vol.

	Number of individuals tested = 50/test						
	Air pur	D.Nour /Air pur	D.Beidha /Air pur	Ghars / Air pur	D. Nour / Ghars	D. Nour / D. Beidha	D. Beidha /Ghars
Active	0	35	19	32	40	33	30
Inactive	49	6	21	8	2	2	4
Sans choix	1	9	10	10	8	15	16

Figures

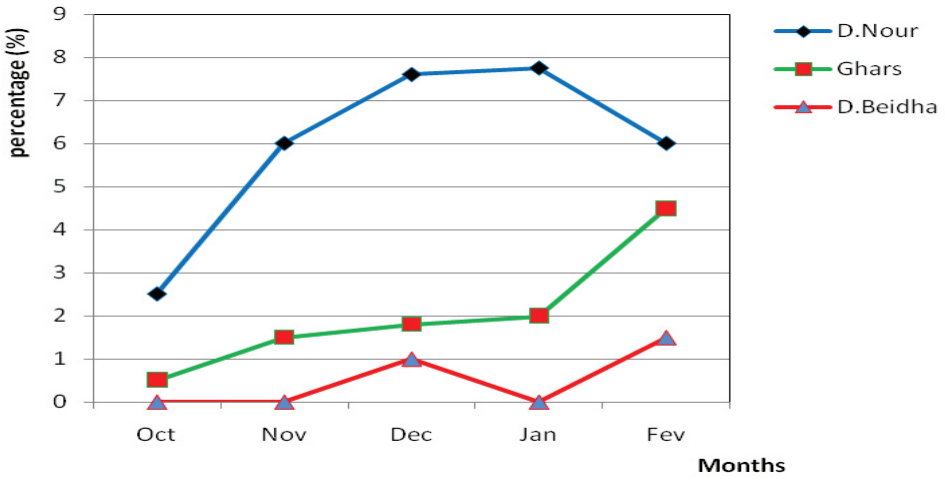

Fig1. Infestation level of the dates by E.ceratoniae in function of the three Algerian dates varieties

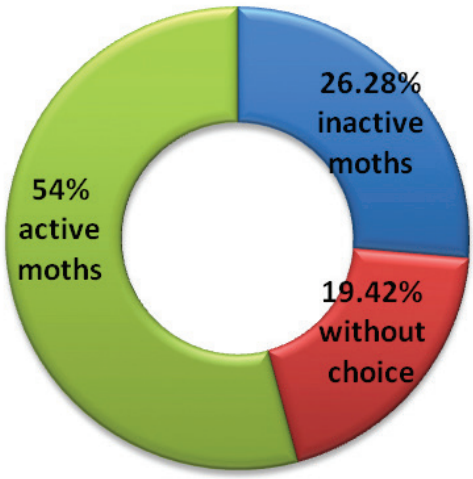

Fig. 2 Behavior of the E.ceratoniae individuals exposed to the odors of three dates varieties in the flight tunnel

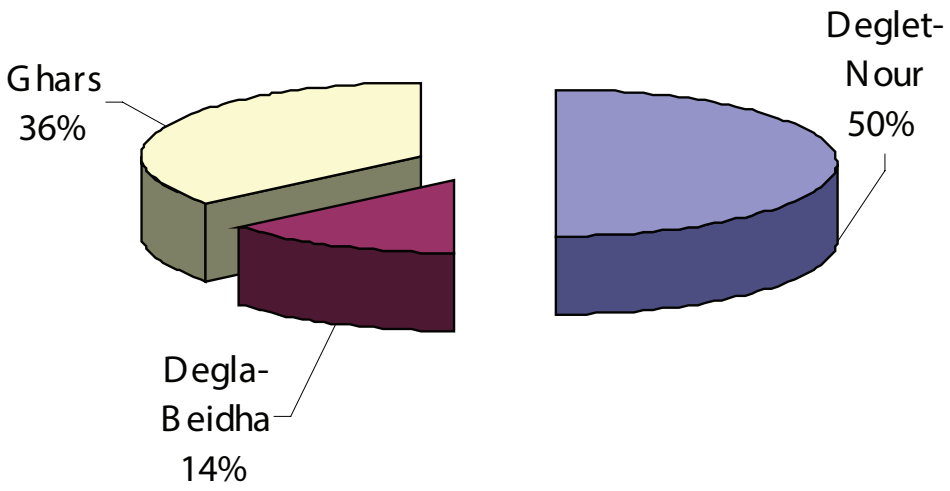

Fig. 3. Distribution of the active E. ceratoniae females as a function of the odors emanate from three cultivars of dates in the flight tunnel.

Fig. 4. Simultaneous representation of cultivars (observations) and variable

Potentials of utilizing biological measures for the management of lesser date moth Batrachedra amydraula in Iraq

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ABSTRACT

The lesser date moth Batrachedra amydraula (LDM) is considered as a key pest attacking fruits in almost all date palm growing regions in Iraq. Larvae begin attacking flowers and bore in to newly formed fruits and move to subsequent stages of fruits development. Biological agents such as the egg parasitoid, *Trichogramma evanescens*,the larvae parasitoids *Bracon hebetor* and biological pesticides such as Bacillus thuringiensis kurestaki and Spinosad were implemented against this pest under field conditions during 2009-2013. Light traps were used for the purposes of monitoring adults emergence and timing of application. All treatments showed significant effect in reducing infestation level of the pest compared to non treated fields. However, effectiveness was varied according to the season, the biological control agents and surrounding environment. The present study was undertaken to investigate the feasibility of using biological measures for controlling this pests at different locations in the country. The bio-pesticide Bacillus thuringiensis was applied singly as a spray for a large scale trial in eight date palm growing provinces with total areas reached up to about 1000ha during 2012. Results showed a variable effectiveness against the pest ranged between 35 to 79% according to site and application time. Same results were reported when the combinations of the bacteria Bacillus. thuringiensis with the egg parasitoid *Trichogramma evanescens* and the larvae parasitoid Bracon hebetors, were tested against LDM.Significant yield increase was observed for all bio agents compared to control.Therefore,these bioagents are suggested to be a safe alternative in any IPM program for the control of lesser date moth in Iraq.

Key word: Biological pesticides, Parasitoids, Pest management, Date palm, Iraq.

INRODUCTION

Iraq is considered as one of the oldest countries cultivating date palms. Palms trees and fruits are subjected to infestation by many key pests which can be found where ever these trees are cultivated in the world including Iraq. However, infestation severity and pest distribution are varied according to pest, host plant and surrounding environment (Al-Baker,1972; Ali,2007; El-Haideri and El-Hafeedh,1986; Zaid, 2002).The most known serous and wide spread economic pests include the lesser date moth *Batrachedra amydraula* Meyrick which can be found in almost all date palm growing areas, the dubas bug *Ommatissus lybicus* Debergevin is intensively found in central region and middle Euphrates Provinces of the country, the date palm borers including

Orycrtes elegance Prell.,*Oryctes agamemnon arabicus,*the long horn stem borer *Jebuseae hammershmitti* Riche and frond borer *Phonopate frontalis* Fahraeus .These borers

can be found everywhere in date palm orchards with the presence of an ecological variation in their distribution according to pest species and the health condition of date palm trees in the intended region. The dust spider mite *Oligonychus afrasiaticus* (Megregor) is a fruit pest which presents a real threat influencing date quality in some seasons. Other pests such as scale insects and some fruit pests are also found in many regions and they are considered as secondary pests however, their status can be changed at any time depending on environmental factors (Abel –Hussain, 1985;El-Haideri and El-Hafeedh,1986;Khalaf et.al.,2013).

The lesser date moth is infesting all date palm varieties with some variations according to variety, region and season. Larvae begin their attack to flowers before fruit setting and continue during the subsequent developmental stages with an intensive increase in Hababoock and Chemri stages feeding on content. The infected fruits become dry and turn red in color from which the insect referred to its name (Hummara).High infestation causes dropping of large quantities of fruits underside the tree leading to big losses in date yield. There are three generations for this pest in most date palm growing areas however, the duration of generation and their peaks are varied according to the region and climatic factors (Al-Fahadawy, 1988;Aziz,2005;Ali et.al, 2010;Al-Safi,1977; El-Juhany, 2010;Kakar et.al.,2010).The chemical insecticides are considered the principle measures used in wide spread application against this pest in most date palm growing regions in the world including Iraq .The organophosphate and carbamite groups were used during the 60^{th} – 80^{th} decades of the past Century, followed by the pyrethroid group and some other recently introduced insecticides (Al-Jboory et. al.,1999; Al-Mhamed, 2001;Bahar et.al., 2010).These insecticides are used either as dust with pollination or as ground and aerial spray (Al-Jboori et.al.,2007;Ali et.al., 2010;AL-Samarraie et.al.,1988;Ba-Angood, 1978). However, the use of wide spectrum insecticide resulted in many negative consequences on human health and environment in addition to the effect on non target organisms including beneficial insects and natural enemies. Therefore, efforts were devoted toward the use of safe or less toxic materials which are called environmentally friend pesticides including biological insecticides such as the formulations of the bacteria *Bacillus thuringiensis* which are considered the most common biological insecticide used against many insect pests. These insecticides were used alone or in combination with other pesticide in an integrated mean for the control of many agricultural pests (Dhoubi et.al.,2007;Sayed et.al,2001).A good results were also obtained for the use of biological insecticide and natural enemies against the lesser date moth and other date palm pests (Ali et .al., 2010; Lysandrou et.al., 2010; Gerling, 2006; Mohammad, 2011; Sayed,2010).Since previous studies indicated that biological agents could be an effective and safe alternative, several field trials were implemented in many of date palm growing regions in Iraq and a satisfactory results were obtained (Mohammad et.al., 2011;2013a;2013b).On another hand some International projects such as Improved Livelihoods of Small Farmers in Iraq through Integrated Pest Management and Organic Fertilization (IRAQ-ICARDA-IFAD PROJECT) and Harmonized Support for Agriculture Development (HSAD) Project in Iraq (USAID-IRAQ-ICARDA PROJECT) in addition to the ongoing national projects are also devoting a large parts of their activities to improve date palm production and protection with the emphasize on the use of natural agents and biological insecticides in an integrated mean for the control of date palm pests. Therefore, the present study was conducted to investigate the feasibility of the use of biological insecticides and natural enemies for the control of lesser date moth in Iraq.

MATERIALS AND METHODS

Information and data obtained from previous studies and from those still ongoing projects concerning the use of biological insecticides and natural enemies against the lesser date moth in Iraq were used as a base line for the present study and for comparison and discussion. A large scale trials for the application of the biological insecticide *Bacillus thuringiensis* (Bt) were also conducted in eight Provinces with a total areas of more than 1300h.during the season of 2012. Provinces included in the trials were Basra, Dewanyia, Babil, Wasit,Najef, Baghdad,Al-Anbar and Dayala.The biological insecticide Bt. was applied at rate of 1.5-3 g/l according to formulation and 6-7 liters of the dilution were used per tree as a spray by ground spraying machines after about two weeks of completion of pollination. Readings on initial infestation in each indented regions were taken before application, and two other readings were also made after treatment .The first one was made after two weeks of application and the seconds reading was conducted after two weeks of the first reading. For each reading three date palm trees were selected randomly in each region within each Province. Three strands were taken randomly from each of the four directions of the tree with a total of 12 strands /tree. Samples were placed in plastic bags and taken to the laboratory for examination. Number of total fruits, infested fruits per stand and percentage of infestation were then calculated. A number of 100 dropped fruits were also collected from under side the trees for examination and recording of percentages of infestation. Total infestation was used for calculation the efficiency of the treatments (Henderson and Telton, 1955). In order to determining the influence of biological insecticide Bt. on date yield, one bunch was cut from each direction of the tree at harvesting time then thenumber of fruits per strand, weight of bunch and the total yield per tree were recorded.

RESULTS AND DISCUSSION

Previous studies on the periodical activity of the lesser date moth using pheromone or light traps indicated the presence of 2 -3 overlapping generation per year in Iraq.The beginning, peak and duration of each generation was varied according to the season and surrounding environment. Results concerning accumulated adults catch in light or pheromone traps in some regions of Middle Iraq during the month of April for some of the past years is presented in (Fig.1).Number of adults was varied with the season due to the variation of surrounding environment and governing climatically factor. Dust storms, rainy thunder storms, wind, extreme decrease in daily temperature and other climatically factors would influence flight activity of adults (Mohammad, 2011; Aziz, 2005). Therefore, trap catch of lesser date moth adults could be considered as qualitative rather than quantitative indicator for the presence of the pest. Accumulated thermal heat units were also used to determine adult emergence and showed that 10% of adults emergence during Spring required an accumulation of 446.06 heat units and most adult emergence occurred during April, which mostly coincide with the flowering stage of date palm trees with an obvious variation between seasons in Iraq,(Al-Dolimey, 2004;Ali,et.al.2010, Mohammad,2011; Aziz,2005).As for the purpose of the present study control measures were implemented depending on actual total infestation in fruits on bunches and in dropped fruits which ranged between 2.6 to 5.5depending on location and time of reading during 2012.Data presented in (Fig.2) indicated that the biological insecticide Bt. was effective in reducing infestation of lesser date moth. However, the efficiency of the control was varied according to region being 79% in Basra and 35% in Al-Anbar. Good results were also obtained in Babil,Baghda,Diwanya,and Wasit Provinces. Other previous field trials showed that the efficiency of the biological insecticide BT. was about 66 - 75 during the seasons of 2009 - 2011.(Mohammad, 2011;Mohammad et.al., 2013a). Trees treated with Bt. Resulted in various yield increase depending on location, cultivar and time of application .The highest percentage of yield increase was more than 100% recorded for Sayr cultivar in Basra and the lowest percentage was 16% recorded in orchards planted with mixed cultivars in Najaf Province during 2012. The reduced efficiency of the biological insecticide which was recorded at Najaf, Dayala, and Al-Anbar Provinces, could be attributed to several factors other than the insecticide including timing of application, effectiveness of the ground spraying machines, the coverage of fruits bunchs,in addition to the skills of the personals involved in application of the biological insecticide. Previous studies also indicated that the use of the biological insecticide Spinosad resulted in control efficiency of about 54 -60% during 2010 and 2011 seasons respectively. Other previous studies indicated that natural enemies such as the egg parasitoid *Trichogramma evanescens* and the larvae parasitoid *Bracon hebetor* were implemented in large scale trials and showed very promising results when applied in proper timing. Results indicated that the efficiency of the egg parasitoid was 68 and 57% during 2010 and 2011 respectively while it was 52 and 64% for the larvae parasitoid during the same seasons respectively (Mahammad,2011;Mohammad et.al., 2011;2013b).These results and the results of the present study showed that biological insecticides and biological agents are promising and safe alternative that can be used in an integrated pest management program for the control of the lesser date moth in Iraq. Since the beginning of the periodic activity of lesser date moth coincides with seasonal flowering of date palm trees which is usually occurs during the month of April with some earliness in southern regions of Iraq, therefore, Survey for fruits infestation should be considered as essential requirement along with adults catch in the light traps, for decision making concerning the control measures against this pest on date palm trees. Farther more, control practices should cover the whole date palm growing areas in order to avoid reinfestation and insure better efficiency of the treatment. A reliable monitoring system is essentially needed to determine proper timing of adult emergence and control decision. Studies on heat units requirement for stages development and adult emergence should take priority in this system (Ahmed and Al-Rubaiee, 2000). Previous studies indicated that the lesser date moth required an accumulated heat units of 626 DD for development of egg and subsequent stages to adults emergence when reared at constant temperature under laboratory conditions (Aziz,2005). However, field studies showed some differences in heat units requirements which were varied according to the season of the study. These variations might be attributed to the length of hibernation periods of larvae and the time required for breaking this hibernation under each certain condition. The establishment of consistent economic threshold for lesser date moth population and infestation is another essential factor which is still need a comprehensive investigation including all aspects of date palm production and protection. Previous studies showed that an economic threshold for lesser date moth was developed based on infestation percentages and number of larvae in 100 fruits sample collected from under side the trees correlated with the input and output requirement of date palm production and protection. The outcome of the study was when percentage of infestation reaches 14.01% and 5.42% and number of larvae reaches 3/100 and 1/100 dropped fruits for the cultivars Zahdi and Khastawy respectively therefore, the infestation level is at the economic threshold and action should be taken (Al-Dolimey, 2004).However, infestation in dropped fruits may not reflect the real infestation because other pest species may be found and feed on dropped fruit (Ahmed and.Al-Rubaiee, 1996). Therefore efforts are still needed to conduct more trials and large scale work in order to establish reliable economic threshold taking in to account the action of physical and

biological factor of the environment including the role of the natural enemies in each date palm growing region and the cultivated variety. The implementation of reliable pest management program will help improving farmer income in addition to reducing environment and health hazards.

CONCLUSSION

All biological control agents and bio- pesticides proved to be promising safe alternative that can be implemented as an integrated control elements against the lesser date moth in Iraq. The egg and the larvae parasitoids are considered as local natural enemies since they were collected from certain regions in Iraq and proved more adapted to the local environment as successful bio-control agents against the lesser date moth. However, the unstable climatically conditions such as the continuous occurrence of dust storms, rain storm and the extreme rise of temperature for several days during spring presenting a real challenge facing the application of bio-control agents against this pest. Timing of releasing bio-control agents or application of bio-pesticides should be decided according to a good sampling procedure that would help in determine a reliable economic threshold which is an essential need for control practice against this pest. The national programs and the international projects, such as Improved Livelihoods of Small Farmers in Iraq through Integrated Pest Management and Organic Fertilization (IRAQ-ICARDA-IFAD PROJECT) and Harmonized Support for Agriculture Development (HSAD) Project in Iraq (USAID-IRAQ-ICARDA PROJECT),which were implemented as a joint activities with the MoA, devoted much efforts of their activities on the use of natural agents and biological insecticides in the integrated pest management practices against date palm pests. All of these projects proved to be effective when applied in a proper timing. More over all biological control means and agents are considered safe environmentally and well integrated elements in any crop management system.

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Figures

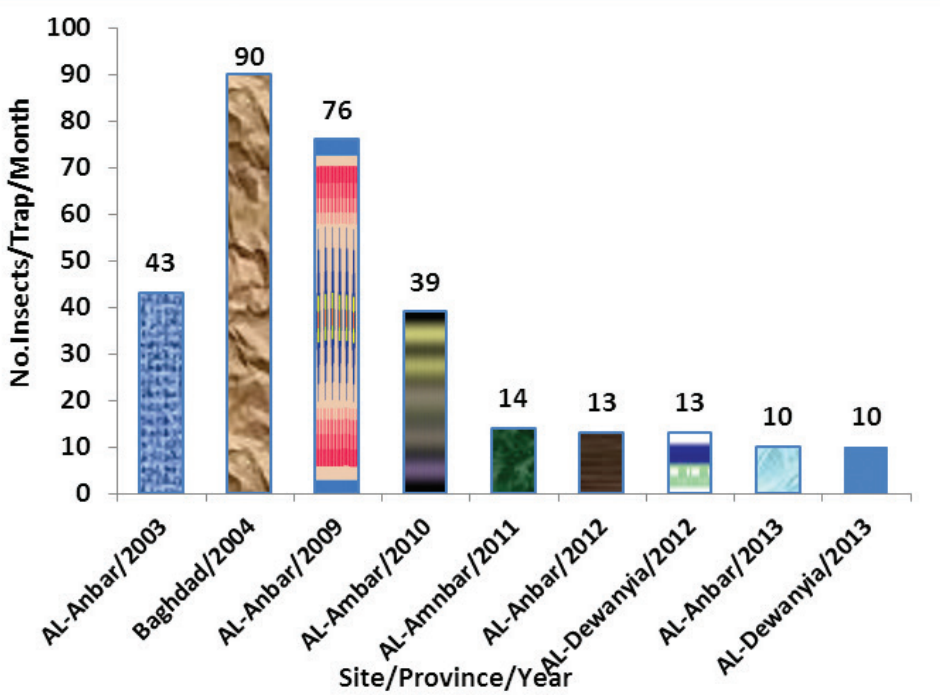

Fig.1 Number of lesser date moth adults caught by light and pheromone traps at different locations during the month of April for the years 2003,2004,2009,2010-2013.

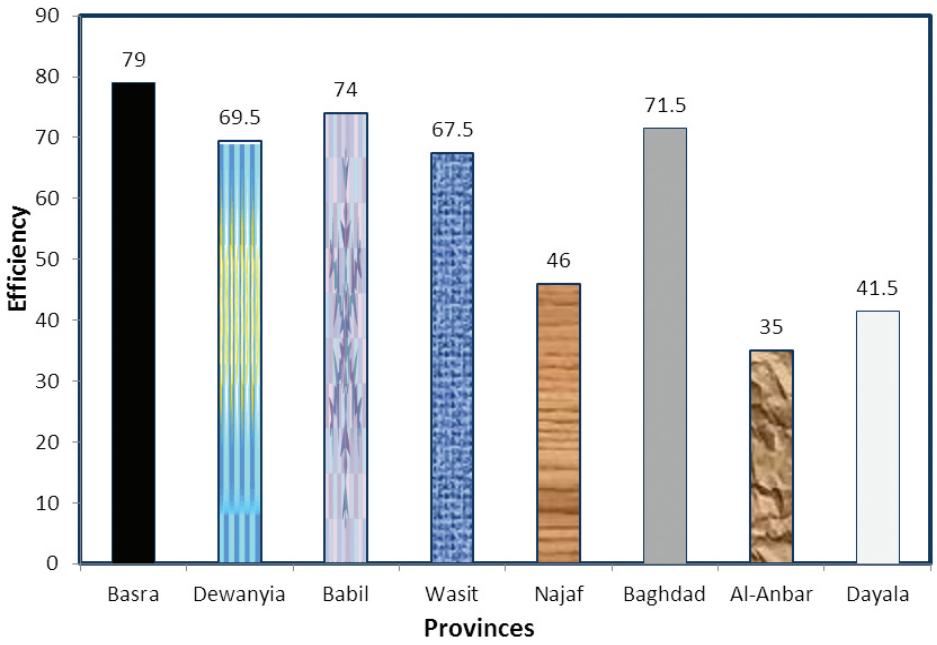

Fig.2 Efficiency of the bio- pesticide Bt. against the lesser date moth at different Provinces in Iraq during 2012.

Occurrence of fungal diseases and their importance in date palm in Sudan

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ABSTRACT

Date palm (*Phoenix dactylifera*) is an important fruit tree in north of Sudan and it was observed to be severely affected by many diseases. This study was carried out to evaluate the incidence and distribution of fungal diseases in date palm in its main producing areas in north of Sudan. A total of 87 date palm orchards (average 82 trees/ orchard) in 29 location in 7 localities in northern states were systematically surveyed during 2009 for the occurrence of fungal diseases. Results revealed that, trunk rot disease caused by T. paradoxa occurred at high levels in Merowe (34.6%), Eldeba (64.8%), Eldamer (96%), Dongla (43.5%) localities and at low level in Abuhamed (1.6%), and East Nile (5.7%). Bud rot (Belaat) disease caused by P. palmivora was observed to occur in Merowe, Eldeba, Dongla and East Nile localities at an incidence of 18.4%, 31%, 33.4% and 17.7%, respectively. Diplodia rot associated with D. phonicum was reported only in Merowe at a level of 18.5 % and in East Nile at 23.1% level. Ganoderma foot rot caused by G. zonatum was observed at Elbawga scheme (100%), Elselaim scheme (24%) and Tangasi Elsouqu (36.8%) in Berber, East Nile and Merowe locality, respectively. An incidence level of 100% of the fusarium wilt disease caused by F. oxysporum was observed only at Tangasi Elrewais (Merowe locality). Further characterization of F. oxysporum using molecular markers is needed for proper identification. In conclusion, Date palm under Sudan conditions is affected by many fungal diseases causing considerable losses in yield. Thus a strategy for proper management of these diseases should be formulated.

Key words: Date palm, fungal diseases, Northern Sudan

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), of the dry type, is one of the major economically cultivated crops in the Northern State of the Sudan, where dates represent about 75% of the exports and also considered a symbol of social status (Dirar, 2003). Date palm is a multipurpose tree that provides food, material for shelter, fuel and timber products. There have been many date palm cultivars grown in northern Sudan and the number of date palm trees in northern Sudan is estimated to be 8 million (FAO, 2005).

Within the last decade, there was a decline in yield of date palm attributed to inadequate cultural practices coupled with infestation of pests and diseases.

However, up to present, only little and very limited work has been done to describe the diseases of date palm in Sudan (Ali,2003; Baghdadi *et al.,* 2003; Idris et al.; 2006). Surveys so far done by plant protection directorate jointly with FAO were incomprehensive and covered very limited areas (Obeid, 1987, and Dadek, 1993). Prior to formulate strategy for the control of date palm diseases, it is essential to identify and determine the economic importance of the diseases. Since it is not known very well the status of date palm diseases in Sudan, particularly fungal diseases, it is paramount important to investigate the occurrence and distribution of fungal diseases in date palm trees in Northern and River Nile States. The present study is an attempt to provide more detail and comprehensive information on the occurrence and distribution of fungal diseases in date palm trees in Sudan

MATERIALS AND METHODS

Surveys for date palm diseases

Surveys for date palm disease were conducted during 2009- 2010 in the main production areas of date palm in northern Sudan. Orchards in different locations in Merowe, Eldebba East Nile and Dongola localities in the Northern State were visited . The survey covered 13 locations in Merowe locality namely; Nori, Abudoom, Tangasi Elsouqu, Tangasi Elrewas, Elgurier, Elbarkal, Elzooma, New amri, Elberkl,Sheba,Elhegaier Elzooma and Jelass. At Eldebba locality the survey covered Hussein Narti, Abudoom Goshabi and New hamadab while in Dongola locality it covered Sortoot, Agaja, Marraga, Skiekh Shareef and Artigasha. In East Nile, the location visited were; Elselaim scheme 1.2.3, Elborgaig scheme, 1.2.3, Bayouda and, Karma Elbalad. In the River Nile State, Atmoor and um Gedai, in Abuhamed locality, ELbawga scheme in Berber locality and Acacia (Jandael) in Eldamer locality .were also surveyed.

In each location, all date palm trees in three randomly selected farmers' orchards were assessed for disease infections. The percentage of disease infections were assessed based on visual symptoms.

Isolation of the causal agents

Plant samples from the infected date palm trees in the surveyed areas were collected in paper bags and brought to the laboratory for identification of the causal agent.

Isolation was done from symptomatic tissues as well as roots, leaves and rachis. Plants material was washed thoroughly under fine spray of tap water to remove adhering soil particles, tissue pieces cut into small pieces of about 0.3 cm, surface disinfected with 0.5% sodium hypochlorite (NAOCL), 2min, rinsed in sterilized distilled waters(SDW) for the same period and left to dry on sterilized filtered paper in flow bench then plated on Petri dishes containing water agar (WA) medium. The growing fungus was then sub cultured on potato dextrose agar PDA medium. The cultures were incubated at 25-30°c. The isolated fungi were identified according to their morphological and cultural characteristics.

Pathogenicity test

The pathogenicity test was only carried out for the fusarium wilt fungus isolated from Tangasi Elrowais. Seeds of the two date palm varieties Barakawi and Mishrig wad khateeb, were surface sterilized for 2 min with 0.5% sodium hypochlorite NAOCL, soaked in SDW and then washed with sterile distilled water before sowing in plastic pots containing sand and clay in a1:2 ratio. Each date palm variety was sown in five pots, five seeds each. The seed lings were kept in nursery and plants were inoculated six month after seedling emergence. 2 ml of *fusarium oxysporum* suspension were prepared and each seedling was dipped in the suspension.

The Fungal culture were grown for 10 days at 25°c on PDA medium the inoculums was prepared by flooding the agar surface of each Petri dishes with 10 ml of SDW and scraping it with spatula. The resulting spore suspension was filtered through four layers of filter paper and the spore concentration was adjusted to 1 +10 spores/ml using hemacytomer.

RESULTS AND DISCUSSION

Disease symptoms and incidence

The most distinctive symptom observed at Tangasi Elrwaise is the appearance on infected trees of whiting of leaves at the second row of heart and white of leaf lets on one side of the rachis, whereas the ones on the other side are green and healthy. A dark brown streak was also observed on petioles and rachis on the side adjacent to the white leaflets. When the affected petiole or rachis was split transversely, a brown discoloration in the xylem tissue was observed indicating *Fusarium spp*. Infection. The Fusarium wilt disease caused by *F.oxysporum* was only observed at Tangasi Elrewais with an incidence of 100% (Table 1).

The most symptoms observed in Hussein Narti, Tangasi Elsouq,, Elgurier,Jelas, abudoom Goshabi, Agja, Marraga, Elselaim, Artigasha, sheikh shareif, umgedi, atmoor,, and Acacia (Jandail)were Several dead trees in which the upper half of the trunks of the affected trees collapsed either falling on the ground or attached to the basal part of the trunk. When a cross section was made on the affected trunk, a brown discoloration starting from the periphery inwards was observed. Another symptoms were black blotches in mid rib varies from spots or blotches. Also there is harmful phenomenon of wilting and drying of bunches shrunken of fruits this phenomenon appear when the fruit changes its color from khalal to rutab stage. These symptoms most likely is attributed to trunk rot disease caused by *T. paradoxa* and/ or its imperfect state *Chalara paradoxa* which were repeatedly isolated from the tissues of affected date palm trunks producing long chains of conidia which fragment readily giving two types.

The trunk rot, Bending head and Black scorch Date bunch fading disorder phenomenon incited by *T. paradoxa* and /or *C. paradoxa* was observed in Tangasi Elsouq, Elgurier, Hussein Narti, Jelass, Abudoom goshabi, Agaja, Marrag, Elselaim, Artigasha, Shiekh sherief, Umgedai, Atmoor and acacia Acacia(Jandail) with an incidence of 74.3, 91.1,100, 46.6, 94.4, 94.9, 80.3, 93.5, 3 4.5, 86.5, 2.2, 0.9 and 96% respectively as shown in Table 1.

A high incidence of quick decline was observed on date palm off –shoots grown in New Amri, New Hamadaab Elbarkel, Marraga and Sortoot with the first symptoms on affected

off-shoots start by drying of the heart and later the leaves around the heart become dry while still keeping the green colour. At a later stage, the heart of the affected trees can be easily removed by hand and the internal tissues become black in colour and have fermented odour. All such symptoms were due to bud rot"Belaat" disease associated with *Phytophthora Palmivoara* which was consistently isolated from tissues of the affected date palm off- shoots at Nori, Abudoom, New amri, New Hamadaab, Elberkl, and Sortoot.

The bud rot disease "Belaat" caused by *P. palmivora* was observed at Elberkal, Abudoom, New amri, New hamadab, Elborgag, Jelas with an incidence of 85.4, 60.8, 87, 93, 70.7, 6.6 respectively (Table 1, 2, and 3).

Most symptoms observed in Sheba, Elgurier, Bayoda, Nori, Elhegaier and Elarak caused by *Diplodia phonicum* were characterized by death of off-shoots either while they are still attached to the mother palm or after they have been detached and planted, while in the leaves of older infected palms the ventral mid portion of the stalks is commonly affected and showed yellowish brown streaks, 15cm to over one meter in length, extending along the leaf base and rachis. Diplodia rot caused by *Diplodia phonicum* was observed, as shown in Table 1, 2, and 3, to occur at Sheba, Elhegaier Elgurier, Elarak, Nori scheme, Bayod and Karma with an incidence of .77.7,54.1,33.1, 41.3, 34.7.24.0 and 67.2%, respectively.

A lot of date palm trees grown in Elbawga scheme Tangasi Elsouqu and Elselaim showed general decline, slow growth and off- colour foliage. In addition, half moon conks (basidiocarps) of the shelf fungus were found attaching to the base of the trunks and this the sign of ganoderma but rot disease caused by the shelf fungus, *G. zonatum*.

Ganoderma butt rot disease was revealed to occur at Elbawga scheme,Elselaim scheme, Tangasi Elsouqu with an incidence of about 100, 93.5 and 36.8 % respectively ((Table 1, 2, and 3). Ganoderma has been known in Elbatywga scheme since a long time ago (personal communication) and it seems that there is a tremendous increase in the incidence of the disease.

This survey revealed the fact that, fungal diseases are the most important diseases widely spread in all areas grown with date palm trees in Sudan in agreement with Zaid *et al.,*(2002) that fungi are the most pathogen found in date palm. Fungi are most likely play an important role in the decline of date palm yield in Sudan. The old plantations, poor cultural practices and the absence of any control measures will eventually aggravate the situation.

Isolation, identification and pathgenicity test of F. oxysporum.

The Fusarium wilt fungus, *F. oxysporum*, was consistently isolated from tissues of affected trees at Tangasi Elrewais. The growth of the isolated fungus on PDA was first white in colour and later developed into pinkish white. The characteristic macroconidia, microconidia and chlamydospores of *F. oxysporum* were observed. *F. oxysporum* produced white and brown spots on the leaves of the inoculated seedlings of both date palm varieties, Wad Khateeb and Barakawi (Plate 16). The inoculated seedlings of Wad Khateeb and Barakawi varieties died after 10 and 21 days, respectively. The morphological and cultural characteristics of the re-isolated fungus from roots of inoculated seedlings were typical to that of *F. oxysporum*.

The identity of the Fusarium wilt fungus was confirmed by the Agricultural Research Centre in Tunisia and the Fusarium Department, University of Sains, Malaysia and ICARDA Syria However, further work is needed for characterization of the *F.oxysporum* using molecular markers.

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Tables

Table 1. Incidence of fungal diseases in different sites in Merowe Locality, Northern State, Sudan, (2009-2010).

Site	Disease	No. of orchards Visited	No. of total trees	No. of infected trees	Disease incidence (%)
Tangasi elrewaise	Fusarium wilt	3	320	320	100
angasi elsouqu	Trunk rot +foot rot	3	440	327+162	74.3+36.8
Elgurier	Trunk rot +diplodia rot	3	362	330+120	91.16+33.1
Nori scheme	Diplodia root rot +Shurnken of fruit	3	470	163+222	34.7+47.2
Abudoom	Bud rot	3	102	62	60.8
New Amri	Bud rot	3	370	322	87.02
Sheba	Diplodia root rot	3	112	87	77.7
Elberkel	Bud rot	3	377	322	85.4
Elzooma	Trunk rot	3	250	130	52
Elmegel	Black scorch	3	222	205	92.3
Elhegeier	Diplodia rot	3	170	92	54.1
Elarak	Diplodia rot	3	290	120	41.3
Jelass	Trunk rot+bud rot	3	150	70+10	46.6+6.6

Table 2. Incidence of fungal diseases in different sites in Eldebba Locality Northern State, Sudan, (2009-2010).

Site	Disease	No. of orchard visited	No. of total trees	No. of infected trees	Disease incidence (%)
Hussien narti	Trunk rot	3	370	370	100.0
Abudoom Goshabi	Black scorch	3	217	205	94.4
New hamadaab	Bud rot	3	115	107	93.04

Table 3. Incidence of fungal diseases in different sites in Dongola and East Nile Localities, Northern State, Sudan, (2009-2010).

Site	Locality	Disease	No. of orchard visite	No. of total trees	No. of infected trees	Disease incidence (%)
Marraga	Dongola	Bud rot	3	66	53	80.3
Agaja	Dongola	Black scorch	3	118	112	94.9
Sheikh shreef	Dongola	Bud rot	3	82	71	86.5

Site	Locality	Disease	No. of orchard visite	No. of total trees	No. of infected trees	Disease incidence (%)
Sortoot	Dongola	Trunk rot	3	42	37	88.09
Artigasha	Dongola	Trunk rot	3	55	19	34.5
Bayod	East Nile	Diplodia rot	3	75	18+17	24+22.6
Elselaim	Eeast Nile	Foot rot+Trunk rot	3	109	102	93.5
Elborgag	East Nile	Bud rot	3	89	63	70.7
Karma	East Nile	Diplodia root rot.	3	64	43	67.2

Table 4. Incidence of fungal diseases in different sites in Abuhamed, Berber and Eldamer Localities, River Nile State, Sudan, (2009-2010).

Location	Locality	Disease	No. of orchards visited	No. of total trees	No. of infected trees	Disease incidence (%)
Um gedai	Abuhamed	Black scorch	3	570	6	2.2
Elbawga scheme	Berber	Foot rot	3	650	650	100
Atmoor	Abuhamed	Bending head	3	210	2	0.9
Acacia Jandeel	Eldamer	Trunk rot	3	520	500	96

Phytoplasma disease in date palm in Saudi Arabia

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ABSTRACT

The date palm, (*Phoenix dactylifera* L.) is one of the most important cash crops in Saudi Arabia, occupying 155,118ha in which 23 million trees produce more than 991,546t of dates annually. A disease called Alwijam (caused by phytoplasma) has lately been affecting date palms in Saudi Arabia. The main symptoms are leaf stunting, yellow streaking and a marked reduction in fruit and stalk size, which leads to failure in fruit production at harvest time. Identifying the associated phytoplasma and a putative vector are important contributions to our knowledge of the disease in Saudi Arabia and can open up a new understanding of such diseases. In order to facilitate this, date palm samples both with and without symptoms of phytoplasma were collected from different locations of Saudi Arabia. The DNA was extracted from the collected plant samples and indexed by a nested PCR reaction using different sets of phytoplasma generic primers P1/P7 and or, R16mF2/R16mR1, to amplify the 16S rDNA region. PCR products were cloned into a pGEM-T easy vector and sequenced. The sequences of the phytoplasma obtained were submitted to GenBank under the accession numbers JQ045567, JQ045570, JQ045571, KC252994, and KC252995. The sequences of 16S rDNA obtained were compared with those of other phytoplasmas in GenBank and the results obtained indicated that there are two types of the phytoplasma infected date palm: 16SrI in Alhasa and 16SrII in other locations in Saudi Arabia.

Key words: Phytoplasma, Date Palm, 16Sr I, 16Sr II, Saudi Arabia

INTRODUCATION

The date palm, (*Phoenix dactylifera* L.), is one of the most important cash crops in Saudi Arabia. The area planted with date palm is 155,118 ha, in which more than 23 million trees produce more than 991,546 t of dates annually (Statistics Book, Ministry of Agriculture, 2011). Alwijam disease has been investigated by very few scientists. The first record of the disease is recorded in a book by Badawi (1945). The disease was recorded in SA by Elbaker (1952) and Nixon (1954). Later, Elarosi et al. (1983) reported that two species of *Fusarium* were always associated with the root of the Alwijam date palm. The symptoms are characterised by a stunting and yellow streaking of the leaves, with fruits and fruit stalks reduced in size (by around 30%). The association of viral, fungal and nematode pathogens with the disease is not consistent (Abdusalam *et al.,*1992. Abdusalam *et al.,*1993. Elarosi *et al.,* 1982). A phytoplasma pathogen was suspected to cause Alwijam-affected palms, following histopathology and antibiotic therapy studies (Abdusalam *et al.,* 1993). This was further supported by El-Zayat *et al.,* 2000, who reported a similar phytoplasma causing lethal yellow coconut palm in Florida. However, Alhudaib et al (2007) were reported that association of a phytoplasma of 16SrI group, *Ca*. P. asteris, with Alwijam in Alhasa, Saudi Arabia

Phytoplasmas are prokaryote organisms of the class *Mollicutes*, which affects more than 700 plant species from tropical to temperate countries (Jones, 2002). They cannot be cultivated *in vitro* and are mainly transmitted by leafhopper or planthoppers of the order *Hemiptera* (Maixner, 2005). Phytoplasmas have been associated with diseases in date palm such as white tip die-back (WTD), slow decline in Sudan in North Africa (Cronjé *et al.,* 2000a. Cronjé *et al.,*

2000b), yellowing in Kuwait (Al-Awadhi *et al.,* 2002), and lethal decline in Texas (Harrison *et al.,* 2002).

In this paper, we report the association of a phytoplasma of 16SrI group and 16SrII group, with Alwijam in Saudi Arabia.

MATERIALS AND METHODS

Sample collection

A survey was done during 2011-2013 in different location of Saudi Arabia (Alhasa, Alkharj, Almadinah, Jouf, Qassim and Riyadh). More than 900 leaf samples were collected of date palm including some that displayed symptoms (Fig 1) and some that did not. Date palm tissue culture product was used in all our experiments as a (healthy) negative control.

DNA Extraction and PCR

The total DNA was extracted from the collected samples using CTAB with ß-mercapto-ethanol (1-2%) (Doyle and Doyle, 1990) method as follows: 200 mg of date palm tissues were homogenized to powder by adding liquid nitrogen and were homogenized using the coffee blender.

Aliquots of final DNA reparations were used as template for a nested PCR (nPCR) assay with phytoplasma 16S rDNA primers P1/P7 and or R16mF2/R16mR1 (Gundersen *et al.,* 1996). PCRs were performed in 25 µl volumes containing 2 µl of DNA template 1 µl of each primer (10 pmol), 2.5 µl of 2.5mM dNTPs, 2.5 µl of MgCl2, 2.5 µl of 10 X Taq polymerase buffer and 1.5 units of Taq DNA polymerase (Invitrogen). First round amplifications were performed in a thermocycler using an initial denaturation at 94 °C for 2min, followed by 35 cycles at 94°C for 45 sec, 55 °C for 1 min, and 72 °C for 2 min, and final extension at 72°C for 10 min. Aliquots of each reaction mixture were analyzed by 1% agarose gel electrophoresis using TBE buffer. The gel was stained in Ethedium bromide and visualized by UV transillumination and photographed (Syngene Bio Imagins, IN Genius). A DNA size marker (1kb DNA marker Promiga, USA) was used to estimate the sizes of the PCR products.

Sequencing and data analysing

Phytoplasma rDNA amplified by PCR using the primer pair P1/P7 was purified on spin columns (QIAquick gel extraction kit; QIAGEN. UK). To determine which group this phytoplasma is located in, the PCR products in each location were purified with from agarose gel as above. The purified PCR products were cloned into a pGEM-T easy vector (Promiga, USA) according to the manufacturers' instructions and then sequenced.

An Agincourt CleanSEQ™ kit was used to clean up the PCR sequencing reaction as follows: Twenty ml of PCR sequencing product were used and placed in another clean tube and 10 ml of magnetic beads were added to the PCR sequencing product. Then 80ml of 80% ethanol was added, mixed and incubated at room temperature for 3 min in a magnetic tray. The liquid was pipetted out of the tube carefully and then 150ml of 80% ethanol were added and pipetted out. Finally the tubes were placed on normal try and 50ml of water was added. Samples were heated at 90 °C for 3 minutes, then half of each sample was loaded on an ABI 377XL automated DNA sequencing instrument, using a 36 cm well to read plates and a 5% Long Ranger (FMC) acrylamide gel. The 16S rDNA sequences of phytoplasmas identified in our study were compared with others in Genbank (Table 1) by BLAST (Altschul *et al.,* 1990). Sequences were aligned and a phylogenetic tree constructed by the program MEGA version 5 (Tamura *et al.,* 2011) using 1000 bootstrap datasets to support the branch values. *Acholeplasma palmae* was used as the outgroups to root the Phylogenetic tree.

RESULTS AND DISCUSSION

Survey and PCR to identify of date palm phytoplasma:

This survey was carried out to determine the occurrence of phytoplasma in different date palm farms in Saudi Arabia. PCR was used to detect the phytoplasma in all the collected samples. PCR using different primers and nucleotide sequences were carried out to identify phytoplasma from the collected samples. All the works was done in Pests and Plant Diseases Unit at King Faisal University.

Phytoplasma rDNA was amplified more than 900 leaf samples. Samples were representative with the 876 bp expected second round PCR amplification. No PCR products were obtained from apparently healthy palms (Fig 2). Fig 3 showed that some of tested samples such as 13, 14 and 18 were negative and that other samples were positive such as 1,2,3,4,5,8,15,16,17 and 19. Also this date showed that some plants were symptomless but gave positive reactions to phytoplasma primers and the nucleotide sequence analysis confirmed.

The obtained data after PCR test as shown that in (Fig. 4), the highly percentage of infection with phytoplasma was in Alhasa and the infection percenage near to 12 %, however the percentages of infection in Alkharj, Riyadh and Qassim were 5.5%, 3.4% and 7.6% respectively, in other side there were no infected date palm samples in Almadinah and Jouf and the percentage of infect was 0% in all.

Nucleotide sequencing and alignment analysis:

Positive samples from different location (Alhasa, Alkharj, Qassim and Riyadh) were sequenced and submitted to

GenBank under accession numbers JQ045567, JQ045570, JQ045571, KC252994, and KC252995. Sequence alignment was carried out for those sequences and from the alignment data we discovered that from the phylogeny tree in Fig 4, the sequence of JQ045567 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Alhasa) was almost all (87%) in a separate cluster to the other sequences identified while the sequences of JQ045571 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Alkharj), JQ045570 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Alkharj), KC252994 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Qassim) and KC252995 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Riyadh) were in same cluster and their identity ranged from 96% to 100%. This data indicates that the isolated phytoplasma from Alhasa may be in different group. Therefore, a comparative analysis of the obtained sequences and other sequences that were available in the gene bank was carried out. To determine the evolutionary relationships among those sequences, we selected 15 sequences for the phytoplasma, some belonged to 16SrI (group I) and others to 16SrII (group II) etc. Sequences are present in GenBank under accession numbers, as shown in Table 1. The data of the phylogeny tree (Fig 5) referred to the sequences of JQ045570, JQ045571, KC252994, KC252995 were in same cluster with 16SrII but only the sequence of JQ045567 (sequence for phytoplasma isolated from *Phoenix dactylifera* from Alhasa) was in the other cluster with 16SrI. These results explained why the identity of JQ045567 was 87%, because it belonged to a different group which was agreed with Alhudaib et al 2007. Taken all together, these results indicated that phytoplasma group II (16SrII) was detected on date palms in different locations in Saudi Arabia (Alkharj, Riyadh and Qassim) at the same time while the only location we detected the phytoplasma group I (16SrI) in Alhasa.

Seemüller et al. (1998) considered phytoplasmas as belonging to the same group if they showed 97% or more similarity between their sequences, while values less than 95% would place phytoplasmas in different groups. LDT and FcoLY type-diseases have been associated with phytoplasma diseases in coconut (Warokka, 2005). El-Zayat et al. (2000), identified a phytoplasma associated with Alwijam disease based on an 87% identity of 16S rDNA with that of the Florida lethal yellows phytoplasma; however, the amount of Alwijam samples where the phytoplasma was detected was not specified, and additionally it was not found in Alwijam affected date palms collected from our surveys. The 16SrI, *Ca. P. asteris* is the only phytoplasma group distributed worldwide and the most diverse in plant and insect hosts (Lee *et al.,* 2000). It has been found in sandalwood in India (Schneider *et al.,* 1993), and safflower and carrot in Israel (Orenstein *et al.,* 1999, Schneider *et al.,* 1993). Results of our experiments extend this to date palms associated in Alhasa but not in other locations in Saudi Arabia. Sequence similarity between the phytoplasma 16SrI date palm PPH (from Alhasa) to those in Table (1) in characterized phytoplasma strains *Ca. P. asteris,* AAY, AY1, MPV, OY Date Palm, BD was 95%-99%; and 51% to FCoLY and LDT. Also less than 87% with the rest of phytoplasma group 16SrII. The phylogenetic tree (Fig. 5) reveals that the phytoplasma identified in date palm is phylogenetically distant from phytoplasmas of 16SrIV group. In our survey a phytoplasma of group 16SrII was detected in date palm. Ca. Phytoplasma aurantifolia group has been recorded from weeds and herbaceous crops in areas of Southern Europe, Africa, New Zealand, Asia, Australia and America (Garnier *et al.,* 1991; Leyva-Lo´ pez *et al.,* 2002; Ghosh *et al.,* 1999; Tran-Nguyen *et al.,* 2003; Tessitori *et al.,* 2005; Tolu *et al.,* 2006). Particularly in the Gulf region, the group has been identified in lime (Zreik *et al.,* 1995), alfalfa (Khan *et al.,* 2001, 2002), sesame (Al-Sakeiti *et al.,* 2005, Esmailzadeh-Hosseini *et al.,* 2007) ; and garden beet (Mirzaie *et al.,* 2007) in Iran. Phytoplasma in date palm showed a 98% identity of 16Sr II and phylogeny results (Fig. 5) clearly support that it is a member of this group. This is the first report of the identification of the 16SrII phytoplasma in date palm, and contributes to the knowledge on the biodiversity of phytoplasmas associated with Alwijam disease in the region.

The results in this study obtained indicated that there are two types of the phytoplasma infected date palm: 16SrI in Alhasa and 16SrII in other locations in Saudi Arabia. Further studies of transmission will be conducted to confirm that vector likes leafhopper and weeds might play the role of etiology of this disease, which will allow the development of more efficient control measures, and reveal new insights into the epidemiology of Alwijam disease.

Acknowledgements

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Table

Table 1: Acronyms GenBank accession numbers of phytoplasma 16S rDNA sequences used to construct the phylogenetic tree

Acronym	Phytoplasma strain designation	RFLP Group	Accession number
PYC	'Papaya yellow crinkle'	16SrII	YI0097
PnWB	'Peanut witches broom'	16SrII	L33765
TBB	Tomato big bud phytoplasma	16SrII	EF193359
WBDL	*'Candidatus* Phytoplasma aurantifolia'	16SrII	U15442
TWB	Tomato witches'-broom phytoplasma (our Sequence)	16SrII	HM584815
AAY	'American aster yellows'	16SrI	X68373
AY	Aster yellows phytoplasma	16SrI	AF222063
AY1	Aster yellows phytoplasma strain AY1	16SrI	AF322644
MPV	Mexican periwinkle virescence phytoplasma	16SrI	AF248960
OY	'Onion yellows'	16SrI	D12569
Date Palm	'Date palm phytoplasma' Old Seq from 2007	16SrI	DQ913090
BD	Barley deformation	16SrI	AY734453
LDT	Coconut lethal decline	16SrIV	X80117
FcoLY	Coconut yellows	16SrIV	U18747
A. laidlawii	*Acholeplasma laidlawii*	as root	M23932
PPH	Date palm from alhasa	This study	JQ045567
KhD	Date palm from Alkharj	This study	JQ045570

Acronym	Phytoplasma strain designation	RFLP Group	Accession number
KhD2	Date Palm from Alkharj	This study	JQ045571
QassimD	Date palm from Qassim	This study	KC252994
RiyadD	Date palm from Riyad	This study	KC252995

Figures

Fig. 1: Symptoms of phytoplasma in date palms

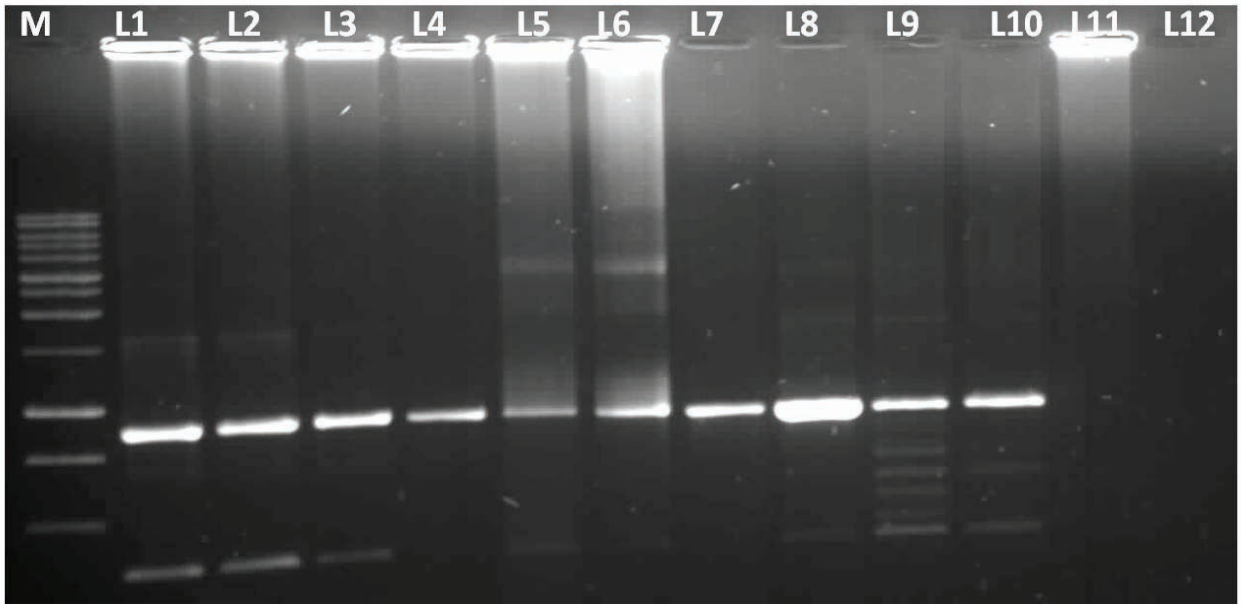

Fig. 2: Agarose gel electrophoresis analysis of nested PCR amplification products using nested primers fU5/rU3. M: 1kb DNA ladder, L1 to L4: positive control, L5 to L10: random date palm samples, L11: negative control and L12: dH2O.

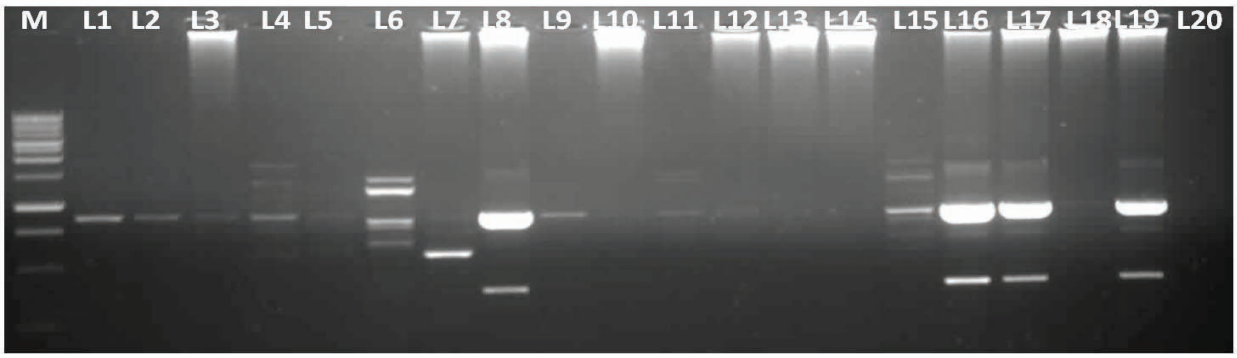

Fig. 3: Agarose gel electrophoresis analysis of PCR amplification products using nested primers fU5/rU3. M: 1kb DNA ladder, L1 to L15: Date palm samples from Alhasa, L16: Infected date palm positive control, L17: Group II positive control, L18: date palm samples from Almadinah, L19: date palm from Alkharj and L20: dH2O negative control.

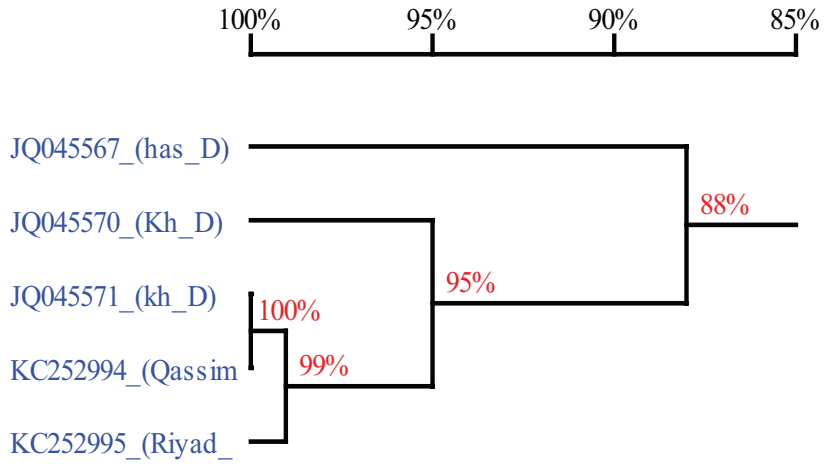

Fig. 4. Phylogenetic relationships of 16S ribosomal RNA gene, for infected date palm using nested PCR with set of primers rU3/fU5.

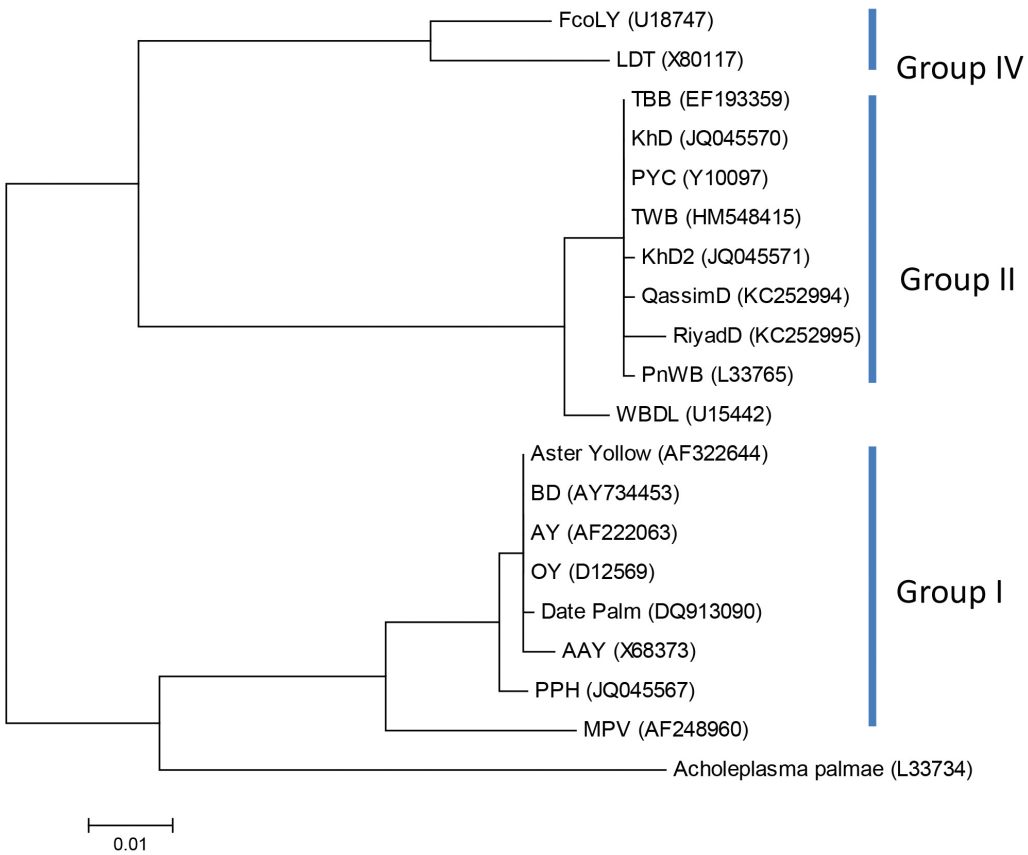

Fig. 5: Phylogenetic relationships tree of 16S ribosomal RNA gene, for infected date palm using nested PCR with the set of primers rU3/fU5 with other sequences obtained from GenBank. The 0.01 bar indicates one nucleotide change per 100 nucleotides.

Using egg parasitoid Trichogramma evanescens and pheromone traps in date palm orchards and in date warehouses to control ephestia spp.

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ABSTRACT

Ephestia cautella, Ephestia figulilella and Ephestia calidella belong to Ephestia genus caused damages to the dates during the storage period which was for about 4-6 months before manufacturing and marketing it. The infestation with Ephestia spp. started in Orchards with the maturation of dates and this infestation transfered to date warehouses if no treatment applied. Therefore, this article concentrated upon releasing Trichogamma evanescens parasitoid in three selected Orchards, the area of each was about 10-15 dunams, these Orchards were in Alrashidia/ Baghdad, Bohruz/ Diyala and Alhur/Karbala In each Orchard 15-18 thousands parasitoid were released /dunam twicelly in autumn and spring. The results illustrated that the percentage of preserving dates while it were on the palm trees were 99.6, 98.5 and 99.2% respectively in the above mentioned Orchards in comparison with 97.5, 97.2 and 98.5% respectively in the control Orchards of the same provinces. To prove that egg parasitoid used was active in controlling Ephestia spp. in the date warehouses too, six date warehouses were selected in the same provinces belonging to date manufacturing and marketing company, in three of them 10 tons of dates were stored in each one, these dates were taken from the Orchards which were treated already with T. evanescens, while in the other three warehouses 10 tons of dates were stored too in each one but the dates were taken from the control Orchards. Then 70000 parasitoids/ warehouse was released in each of the first three date warehouses, then the same numbers were released after two weeks, in each of these three warehouses in addition to nine pheromone traps for each Ephestia spp. hanged, while the control date warehouses left without treatment with parasitoid but pheromone traps hanged too for monitoring. The results revealed that the average percentages of disinfesting dates in the first three warehouses were 99.5, 98.6 and 98.9% respectively in comparison with 84.1, 87.7 and 90.7% respectively in the control warehouses. Furthermore, the results showed that the mean number of the three Ephestia spp. were 0.5, 0.3 and 0.2 insect/trap/ month in the date warehouse of Baghdad and 0.3, 0.4 and 0.2 insect/trap/month in the date warehouse of Diyala and 0.6, 0.2 and 0.1 insect/trap/month in the date warehouse of Karbala in comparison with 17.0, 13.8 and 6.0 insect/trap/month for control warehouse of Baghdad. 16.3, 8.3 and 3.0 insect/ trap/month for control warehouse of Diyala and

finally 13.3, 6.0 and 2.0 insect/trap/month for control warehouse of Karbala. In Conclusion these results are encouraging and using this biological control agent with pheromone traps could be essential to control Ephestia spp. in Orchards and date warehouses instead of chemical insecticide and methyl bromide within IPM program.

INTRODUCTION

Iraq is the oldest countries in the world in date palm plantation . the date palm plantation recognized in Mesotopemia before four thousand years (Hussain and Esmail, 2007, Abdul – Hussain, 1985 and Al – Haidari, 1979). The importance of date palm isnot as dates production only but it also important for improving environment and for protecting citrus trees from high temperature in summer and low temperature in winter in middle and south of Iraq (Hameed, 2002 and AL-Taie, 2001). Till recently Iraq was determined as the main country in producing, packaging and exporting dates internationally (AL-Anbaki, 2007). The main problem facing Iraqi dates trade is its infestation with insect pest mainly *Ephestia* spp., the infestation appeared to be started in the Orchards and transfered with dates to the date warehouses because of the suitable condition in these warehouses for their reproduction (AL-Taweel and Al-Jboory, 2007 and Hameed *et al.,* 2001) The Larvae fed inside date on the flesh which resulted in date become unsuitable for human consumption (Ahmed, 1998), previously to control insect pest in dates warehouses Methyl Bromide was used as fumigant because it was effective in killing most insect stages but this fumigant (Methyl Bromide) appeared to be ozone depleting agents (Marcotte, 1993 and Leesch *et al.,* 1992), therefore, it was prevented since 2005 in developing countries and it will prohibited by 2015 in underdeveloping countries according to Montreal Protocol (Ross and Vail, 1993). Therefore, scientist in Iraq and other countries started to look for new technology which should be acceptable nationally and internationally for date disinfestation, one of these methods is using biological control agents specifically egg parasitoid *Trichogramma evanescens* and pheromone traps for dates disinfestation in Orchards to minimize the percentage of dates infestation with *Ephestia* spp. larvae and using the same biological agents and pheromone traps in dates warehouses after transfering dates from these Orchards and to keep the infestation as low as possible and the manufacturing dates accepted for human cosumption.

MATERIALS AND METHODS

Mass Rearing of Egg Parasitoids Trichogramma evanescens.

Sitrotroga cerealella and *Ephestia cautella* eggs were used as host to produce sufficient numbers of the egg parasitoid (Alrubeai *et al.,* 2005)

Effect of Releasing Egg Parasitoids and Pheromone Traps on the Percentage of Date Infestation in the Orchards.

Three Orchards, each of 10 – 15 donums were selected for this study, these Orchards were located in Alrashidia / Baghdad, Bohruz / Diyala and alhur Karbala . In each Orchard 15 – 18 thousands parasitoids were released/ donum (As pupae inside their host eggs) twicelly in autumn and spring because at these times *Ephestia* spp. (*E. cautella, E. figulilella and E. calidella*) found at their maximum density (Hameed *et al* ., 2011). Moreover, four pheromone traps were used / donum for each *Ephestia* spp. in the Orchards treated with the egg parasitoids while one trap was used for the control Orchards for monitoring. Furthermore, about 125-150 date fruits were collected from each date palm tree selected randomly (Fifty date palm trees) from treated and control Orchards at the time of harvesting date fruits at the begining of November 2012 . The date fruits samples kept in polyethylene bags separatly and transfered to the laboratory for inspection and the percentage of infestation with different *Ephestia* spp. stages was recorded.

C) Effect of Field Treatment (in b above) on the Percentage of Infestation of Date Fruits Stored in the Date Warehouses.

Two date warehouses each of 4 x 10 x 6 meters were prepared / each province mentioed in (b) above, in the first warehouse ten tons of dates harvested from treated Orchard in (b) above was stored while in the second warehouse ten tons of dates harvested from control Orchard in (b) above was stored . Nine pheromone traps for each *Ephestia* spp. were hanged in the first warehouse of each province while one pheromone trap was hanged in the control warehouse of each province. Furthermore, seventy thousands egg parasitoids (*T. evanescens*) were released within the first week of storing dates and after weeks from the first release another seventy thousands egg parasitoids were released too in the first date warehouse of each province . while the second date warehouse of each province left without any treatment. The experiment continued for six months starting from November / 2012 till April / 2013. Moreover, to study the efficacy of *T . evanescens* (the egg parasitoid), two hunderd sterilized eggs of the host with UV were placed

in petri dish and placed in each treated date warehouse monthly, the percentage of parasitizim was recorded . Finally two hunderd Kilograms of dates were selected randomely from each treated warehouse for each province and packed in polyethylene pags, transfered to the laboratory for inspection . The same amount of dates also inspected from control date warehouses for the same provinces.

RESULTS AND DISCUSSION

The results of table (1) illustrated significant differences ($P < 0.05$) in the percentage of infested dates collected directly from the date palm tree of the Orchards treated with the egg parasitoids and pheromone traps in comparison with control Orchard . Furthermore, table (1) also showed that the average of infestation in the Orchard treated with the *T. evanescens* and pheromone traps in Baghdad, Diyala and Karbala provinces were 0.4, 0.2 and 0.8 % in comparison with 2.5, 2.8 and 1.5% for the control Orchard for the same provinces respectively . These results were agreed with Strong and Morrison (1980) results and Alrubeai *et al*. (2003) in their experiments in which they used egg parasitoid *T . evanescens* to control insect pest, Furthermore the average number of *E. cautella*, *E. figulilella* and *E . Calidella* captured by pheromone traps was reduced to 2.2, 1.7 and 1.2 in the treated Orchard in comparison with 37.7, 52.0 and 24.7 in the control Orchard respectively . This could be as a result of releasing egg parasitoid in the Orchards which act as an agents to reduce *Ephestia* spp. populations in the treated Orchards.

The results represented in tables 2, 3 and 4 showed also significant differences ($P< 0.05$) in the percentage of infested dates in the date warehouses treated with *T. evanescens* and pheromone traps in comparison with control date warehouses in Baghdad, Diyala and Karbala . The percentage of infested dates in the treated warehouses were 0.5, 1.4 and 1.1% in comparison with 15.9, 12.3 and 9.3% in control date warehouses respectively . These results agreed with Thrope and galen (1985) and with Grille and Basso (1995) who stated that the activity of egg parasitoids increased if the warehouses areas decreased, also they found that the temperature in warehouses was suitable for the egg parasitoid activity (21 – 25 ^{0}C) which was approximatly equal to temperature in the date warehouses, these results also agreed with Ahmad and Ali (1989) who found the importance of pheromone traps in reducing *E. figulilella* population density in the field if the pheromone trap used as an agents to control this pest within Integrated Pest Control Program. Furthermore, the results of the same tables (2, 3 and 4) showed that the average number of *Ephestia* spp. (*E. cautella, E. figulilella* and *E . calidella*) in the treated date warehouses were 0.5, 0.3 and 0.2 insect / trap / month for Baghdad; 0.3, 0.4 and 0.2 for Diyala and 0.6, 0.2 and 0.1 for Karbala in comparison with control date warehouses were 17.0, 13.8 and 6.0 insect / trap / month for Baghdad; 16.3, 8.3 and 3.0 insect / trap / month for Diyala and 13.3, 6.0 and 2.0 insect / trap / month for Karbala. These results are very encouraging and showed the importance of primary treatment of the Orchard with egg parasitoid and pheromone trap in reducing the infestation rate of dates and average number of insect / trap / month. On the other hand the results showed that 85% of host eggs which placed in the treated date warehouse was parasitized by the egg parasitoid *T. evanescens* which mean that the egg parasitoids were capable to find its host egg in the date warehouse and these result support the above results which showed reduction in the average number of insect captured per trap per month .

In conclusion, the results of this investigation showed the possibility to disinfest dates in the date warehouses before preparing it for manufacturing and packaging it for exportation instead of Methyl Bromide using the biological control agents and the pheromone traps.

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Tables

Table 1. Number of Date Fruits Inspected (Which Directly Collected from Date Palm Trees) from the Orchards Treated by the Parasitoid *Trichogramma evanescens*, Pecentage of Infested Date and Average Number of *Ephestia* spp. captured by Pheromone Traps in the Date Orchards of Baghdad, Diyala and Kabala Provinces.

Orchard Location	Nos.of Date Fruits Inspected	%Non infested and infested Dates*		Average Nos.of Ephestia spp. Captured by Pheromone Traps**•		
		Infested	Non-infes ted	E. cautella	E.figulilella	E.calidella
Baghdad AlRashidia						
Treated	6300	0.4	99.6	3.2	2.1	1.2
Non-treated	6800	2.5	97.5	62	87	23
Diyala/Buh-ruz						
Treated	7250	0.2	99.8	1.2	1.7	1.8
Non-treated	8200	2.8	97.2	28	41	19
Karbala/Alhur						
Treated	7300	0.8	99.2	2.1	1.2	0.7
Non-treated	6900	1.5	98.5	23	28	32

Orchard Location	Nos.of Date Fruits Inspected	%Non infested and infested Dates*		Average Nos.of Ephestia spp. Captured by Pheromone Traps**•		
		Infested	Non-infes ted	E. cautella	E.figulilella	E.calidella
Average for Treated	6950	0.5	99.5	2.2	1.7	1.2
Average for Non-treated	7300	2.3	97.7	37.7	52	24.7

- * T-test showed significant differences between infested and noninfested dates (P< 0.05) collected from treated Orchard and nontreated Orchard. Calculated T value=3.666 and Tabulated T value= 3.182
- ** T-test showed significant differences (P<0.05) between average number of *Ephestia* spp. captured by pheromone traps in treated Orchard and nontreated Orchard with the Egg parasitoid .. Calculated T value=4.451 and Tabulated T value= 3.182.
- Four pheromone traps/donum for each *Ephestia* spp. for treated Orchard and one pheromone trap/donum for control Orchard.

Table 2: Controlling *Ephestia* spp. in Date Warehouse Using Egg Parasitoid. *T. evanescens* and Pheromone Traps in Baghdad Province / Alshaljiya .

Date of Inspection	No. of Kg. of Date Fruits Inspected		% Non infested and infested Dates •				Average No. of *Ephestia* spp. Captured by Phoromone Trap*••					
			Treated warehouse		Control warehouse		Treated ** warehouse			Control*** warehouse		
	Treated warehouse	Control warehouse	Non infested	Infested	Non infested	infested	(1)	(2)	(3)	(1)	(2)	(3)
Nov./2012	200	200	99.6	0.4	97.8	2.2	0.2	0.3	0.5	4	8	4
Dec./2012	200	200	99.7	0.3	94.2	5.8	0.3	0.1	0.3	8	12	7
Jan./2013	200	200	99.3	0.7	87.5	12.5	0.5	0.3	0.2	13	14	6
Feb./2013	200	200	99.8	0.2	81.4	18.6	0.3	0.6	0.0	17	10	5
Mar./2013	200	200	99.2	0.8	73.8	26.2	0.9	0.4	0.1	28	18	8
April/2013	200	200	99.4	0.6	70.0	30.0	0.8	0.0	0.0	32	21	6
Total	1200	1200	597	3.0	504.7	95.3	3.0	1.7	1.1	102	83	36
Average	200	200	99.5	0.5	84.1	15.9	0.5	0.3	0.2	17.0	13.8	6.0

- * *Ephestia* spp. : (1) *E. cautella*; (2) *E. figulilella* and (3) *E. calidella* .
- ** Nine pheromone traps for each *Ephestia* spp. were hanged in the treated date warehouse (Mass trapping).
- *** One pheromone trap for each *Ephestia* spp. was hanged in the control date warehouse (Monitoring)
- T-test showed significant differences (P<0.05) between the average percentage of infested dates in the treated date warehouse in comparison with control date warehouse, Calculated T value= 3.402, Tabulated T value = 2.441.
- •• T-test showed significant differences (P<0.05) between the average nos. of captured *Ephestia* spp. by pheromone traps in the treated date warehouse in comparison with the Control date warehouse, Calculated T value= 12.590, Tabulated T value = 2.441.

Table 3: Controlling *Ephestia* spp. in Date Warehouse Using Egg Tarasitoid . *T. evanescens* and Pheromone Traps in Diyala Province/Buhraz.

Date of Inspection	No. of Kg. of Date Fruits Inspected		% Non infested and infested dates •				Average No. of *Ephestia* spp. Captured by Phoromone Trap*••					
			Treated warehouse		Control warehouse		Treated ** warehouse			Control*** warehouse		
	Treated warehouse	Control warehouse	Non infested	infested	Non infested	infested	(1)	(2)	(3)	(1)	(2)	(3)
Nov./2012	200	200	98.7	1.3	98.6	1.4	0.3	0.8	0.5	6	8	2
Dec./2012	200	200	97.4	2.6	93.8	6.2	0.2	0.9	0.3	10	6	5
Jan./2013	200	200	98.9	1.1	92.2	7.8	0.4	0.1	0.0	12	8	2
Feb./2013	200	200	98.8	1.2	88.3	11.7	0.5	0.1	0.0	16	6	3
Mar./2013	200	200	99.0	1.0	81.8	18.2	0.3	0.4	0.2	26	13	2
April/2013	200	200	98.9	1.1	71.5	28.5	0.2	0.1	0.0	28	9	4
Total	1200	1200	591.7	8.3	526.2	73.8	1.9	2.4	1.0	98	50	18
Average	200	200	98.6	1.4	87.7	12.3	0.3	0.4	0.2	1.3	8.3	3.0

- * *Ephestia* spp. : (1*) E. cautella*; (2) *E. figulilella* and (3) *E. calidella* .
- ** Nine pheromone traps for each *Ephestia* spp. were hanged in the treated date warehouse (Mass trapping).
- *** One pheromone trap for each *Ephestia* spp. was hanged in the control date warehouse (Monitoring)
- T-test showed significant differences ($P<0.05$) between the average percentage of infested dates in the treated date warehouse in comparison with control date warehouse, Calculated T value= 3.402, Tabulated T value = 2.441.
- •• T-test showed significant differences ($P<0.05$) between the average nos. of captured *Ephestia* spp. by pheromone traps in the treated date warehouse in comparison with the Control date warehouse, Calculated T value= 12.590, Tabulated T value = 2.441.

Table 4: Controlling *Ephestia* spp. in Date Warehouse Using Egg Parasitoid. *T. evanescens* and Pheromone Traps in Karbala Province/Al hur.

Date of Inspection	No. of Kg. of Date Fruits Inspected		% Non infested and infested Dates •				Average No. of *Ephestia* spp. Captured by Phoromone Trap*••					
			Treated warehouse		Control warehouse		Treated ** warehouse			Control*** warehouse		
	Treated warehouse	Control warehouse	Non infested	infested	Non infested	infested	(1)	(2)	(3)	(1)	(2)	(3)
Nov./2012	200	200	98.9	1.1	98.3	1.7	0.4	0.5	0.1	5	3	1
Dec./2012	200	200	98.6	1.4	96.5	3.5	1.2	0.3	0.2	6	8	2
Jan./2013	200	200	98.5	1.5	92.8	7.2	0.6	0.1	0.5	10	7	3
Feb./2013	200	200	98.8	1.2	88.2	11.8	0.7	0.0	0.0	15	8	1
Mar./2013	200	200	99.2	0.8	86.8	13.2	0.4	0.0	0.0	18	2	2
April/2013	200	200	98.5	1.5	81.8	18.2	0.2	0.0	0.0	26	8	3
Total	1200	1200	592.5	7.5	544.4	55.6	3.5	0.9	0.8	80	36	12
Average	200	200	98.9	1.1	90.7	9.3	0.6	0.2	0.1	13.3	6.0	2.0

- * *Ephestia* spp.: (1) *E. cautella*; (2) *E. figulilella* and (3) *E. calidella* .
- ** Nine pheromone traps for each *Ephestia* spp. were hanged in the treated date warehouse (Mass trapping).
- *** One pheromone trap for each *Ephestia* spp. was hanged in the control datewarehouse (Monitoring)
- T-test showed significant differences ($P<0.05$) between the average percentage of infested dates in the treated date warehouse in comparison with control date warehouse, Calculated T value= 3.402, Tabulated T value = 2.441.
- •• T-test showed significant differences ($P<0.05$) between the average nos. of captured *Ephestia* spp. by pheromone traps in the treated date warehouse in comparison with the Control date warehouse, Calculated T value= 12.590, Tabulated T value = 2.441.

The influence of Diatomaceous Earth (DE), (Agripower Silica as composed of the skeletal remains (diatoms) of freshwater algae (species *Melosira Granulata*)) on the growth and development of Date Palms, grown under moderately saline irrigation water in the Riverland of South Australia.

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ABSTRACT

The South Australian Mallee region is characterised by alkaline soils. The site where the date palms are grown in this trial are from a moderately saline backwater of the Murray River where irrigation EC can be as high as 2600Ec Ds/m. Published research has indicated that silica is a very important nutrient in date palm production and that reduction in plant silica levels resulted in slower growth rates . The aim of this project was to investigate the role that increasing silica applications (silica as diatoms of Melostra granulata) would have on the growth and development of young date palms and if previously published research could be replicated under Australian growing conditions. Applications of the product were applied to Barhee date palms at the rate of 2, 4 and 8kg per palm tree in October 2012. Tissue test data revealed less Si where the Agripower Si product had been applied irrespective of treatment application in comparison to the control. It is proposed that this decrease in plant tissue levels is a function of a growth dilution effect. What was noticeable was that where the product was applied mean frond and leaflet length were significantly increased in comparison to untreated control palms. Mean leaflet length increased from 37.04cm Control to 40.13cm (2kg/palm), 41.04cm (4kg/palm) and 41.58cm (8kg/palm) and mean frond length from 2.52m (Control), 2.64m (2kg/palm), 2.67m (4kg/palm) and 2.68kg (8kg/palm). The significance of this data was that treated palms were visibly larger than untreated control palms within the first growing season that

the product was applied. Further applications of Si were made in September 2013 for continuation of assessment of long term implications of Si applications as Agripower DE on the growth and production of date palms in this region.

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INTRODUCTION

Date palms have been grown in Australia since the late 1800's yet it is has only been in relatively recent times has the crop been looked as a potentially significant horticultural crop in Australia. As a result the technical understanding of production and suitable varieties relies heavily on the experience of those in the Northern Hemisphere and extension and application of this data into Australian growing conditions. As a result the agronomic knowledge of specific issues in managing Date Palms in Australia, and the variance of application of overseas data for Australian growing conditions are still very much based on learning to adapt overseas information. This has meant determining the significance of overseas data and screening its' application to Australian growing conditions.

Matichenkov and Bocharnikova (2006) noted that historically date palms grew in oases that were related to a geological depression and artesian waters. These regions were characterised by silicon rich waters due to the concentration of mobile silica in the geological depression zones. Their conclusion was that date palms require high amounts of silica and in further studies determined that plants irrigated with desalinated water were deficient in silica nutrition. Fruit analysis determined that the maximum total of Si could be found in the epidermal tissue of the clingstone (0.81-1.51% from dry mass) and lower amounts in the fruit stem, clingstone core and pulp. They also noted that as Si levels dropped so did fruit sugar content as did the growth of young palms. With increasing plantations of date palms grown on water significantly different from the composition of the oases where they were originally grown, Si could play a significant role in plant nutrition.

From being a plant found around oases throughout the Middle East today the date palm is commercially grown on reclaimed, desalinated, bore and river water across the globe. Therefore the chemical composition of the water is significantly different to that which the plant has evolved on and this variance could create issues in the growth and development of date palms in a number of environments.

Silica is such a large percentage of the mineral matter of the earth's crust by weight (27.7%) (Hausenbuiller,(1985). With such a high percentage of silica in the earth's crust, the role of silica in plant nutrition has been regarded more widely as a curiosity to researchers; at a field level applications of silica for crop nutritional purposes are largely ignored. However in recent years there has been a renewed interest in the potential role of Silica in plant nutrition.

Silica has been well documented as a plant nutrient and oats can contain 1% Si as dry material (Leeper 1967). This is significantly more than K in dry matter at 0.6%. Leeper notes in his book 'Introduction to Soil Science' which has been a foundation book of young soil scientists since it was first published in 1948 and edited over the next 30 years was that a soil with a good supply of primary silicates can remain chemically rich over many years of cropping. An interesting note in this book on page 170 states that 'The conclusion is rather that the primary minerals can guarantee the chemical fertility. The experiment is so striking that it is strange that this section of the knowledge of soils has been so neglected. The experiment also shows the powerful weathering properties of acid clay.' Is it possible that with Si being such an abundant element that it has been traditionally regarded as an element that does not gain a lot of attention in traditional plant nutritional management plans? The documented variance in Si levels between different plant species also makes conventional recommendations more difficult than with nutrients such N, P and K.

It is also interesting to note that aluminium toxicity in soils while being dependant on solubility at low soil pH but also on the nature of the clay soil that is being acidified. It has been noted that the sesquioxidic clays provide more aluminium than the siliceous clays and hence a higher degree of toxicity under acidifying conditions. The significance of this should not be lost in the potential of Si to alleviate Al toxicity under acidifying soil conditions. With increasing use of recycled water and acidification of drip zones issues around aluminium toxicity and potential management considerations may need to be considered. It is in these areas that the use of silica may be beneficial.

Leeper further notes that the monocots absorb silicon in large amounts with up to half of the ash being Silica dioxide. Si has been documented in increasing resistance to pathogens such as blast in rice(Datnoff et al 1997) and powdery mildew in cucumber (Miyake and Takahashi 1982).Si has also been found to prevent lodging in rice and it is perhaps this role that has the greatest application in world wide applications.

Jones and Handreck (1967) note that importance that Si plays in alleviating Mn toxicity. This may play an important role where acidic soil conditions are required but Mn toxicity symptoms are an issue. Applications of Si may be useful in reducing plant available Mn and reducing toxicity risks. West (et al) 2007, note that while Si is not noted as an essential mineral element for plant growth it has many beneficial effects on plant performance. This stems from increased resistance to fungal diseases, improved mechanical stability of leaf and blades and improved water stress. They further note that there is great variation in uptake of Si in plant species and that it is not unrealistic to assume that responses to soil applied Si in one species do not mean that similar responses would be expected in other species.

Ahmed et al (2012) note that Si is able to help plants withstand the adverse effects of drought and improve plant water use efficiency. It is proposed that the mechanisms to improved salinity tolerance in plants as a result of Si were increased photosynthetic activity and ultra-structure of leaf organelles. In their work on drought tolerance of wheat they determined that while a single trait cannot make a plant resistant to water stress, the beneficial effects that Si would play for screening drought resistant genotypes. In a world facing heat waves and shortages of irrigation water this work may prove to be significant in our application of Si to agricultural crops

Silicon (Si) plays a significant role in imparting biotic and abiotic stress resistance (Ma et al, 1989) and enhancing growth and yield, especially in accumulator species (Street-Perrot and Barker, 2008). While there have been numerous studies, some specific examples include Si increasing resistance to pathogens such as blast in rice (Datnoff et al, 1997) and powdery mildew in cucumber (Miyake and Takahashi, 1982). Si has been found to prevent lodging in rice and it is perhaps this role that has the greatest current application in worldwide applications.

Si deficiency in soil is now recognized as being a limiting factor for crop production, particularly in soils that are deemed to be low or limiting in plant available Si and for known Si-accumulating plants (Ma and Takahashi, 2002).

The plant available Si of a soil is reliably measured through an extraction procedure using a calcium chloride solution. Calcium chloride extracts the easily soluble Si and has been shown to correlate well with yield increases (Berthelsen et al, 2001; Haysom and Chapman, 1975). Critical limits and ranges have been reported (Narayanaswamy and Prakash, 2009) for the $CaCl_2$ extractant method on soils. They determined that 43ppm was the critical soil level for this extraction method.

The aim of this trial is to investigate the potential use of Si in the mallee soils used for date palm production and if published research work on the role of Si in date palm growth can be extended into Australian growing environments.

MATERIALS AND METHODS

Site

The date palm trial site is located at the property of Dave and Anita Reilly in the Gurra region of the Riverland in South Australia. The property is irrigated out of the Gurra Lake which is a backwater from the Murray River. Due to its location water is significantly more saline than irrigation water taken straight out of the river system. Irrigation water has been as high as 5000EC dS/m but with the breaking of the drought and increased flows in the river system, irrigation water salinity levels have dropped to between 1200-1800 EC dS/m in recent seasons.

The local climate is associated with hot dry summers and mild winters with rainfall averaging 245mm/pa. Due to the nature of storm events, rainfall is highly variable and over the past 10 years annual rainfall events have ranged from 83-435mm/pa.

The soil at this property is a calcareous sandy clay loam with clay subsoils at depth. Irrigation is undertaken with drip irrigation with drippers based around each palm. Dripper output is 25L/hr with 3 drippers per palm. The large output drippers enable soils to be filled to field capacity and manage high algal content associated with water in this area. The larger outputs also enable better manage for the high colloidal content of the irrigation water.

Treatments

Applications of the Agripower Si material were banded around the base of each *Barhee* date palm at rates of 2, 4 and 8kg per palm. As the site is used for commercial production full rows of each treatment were implemented for incorporation with minimal disruption into the overall farm management operation.

Agripower Si was applied on the 14/9/12 and again on the 4/9/13. Measurements of soil nutrient status, tissue tests and plant phenology were undertaken over the 2 growing seasons.

RESULTS

A complete analysis of the Agripower Si sample what was to be applied in the trial site and a pre-treatment soil test were undertaken to determine starting point soil fertility and also the chemical composition of the Si to be applied in this soil test

Table 1: Technical Information on Product: AgriPower Silica Raw Material: Diatomite A typical analysis of this mineral is given below:

Typical Analysis	
Calcium	1.5%
Iron	5.9%
Magnesium	1.05%
Potassium	0.07%
Zinc	19ppm
CEC	52cmol/kg
Soluble Si	1,212ppm
pH	8.1

Table 2: Soil test results from trial site, prior to application of AgriPower Silica

Element	Result	Units
pH (1:5)	8.7	
Electrical Conductivity (1:5)	0.16	mS/cm
Organic Carbon	0.3	%
Nitrate – N	4	mg/kg
Phosphorous (BSES)	41	mg/kg
Phosphorous (Colwell)	18	mg/kg
Phosphorous (Olsen)	11	mg/kg
Potassium (exchangeable)	431	mg/kg
Calcium (exchangeable)	4858	mg/kg
Magnesium (exchangeable)	302	mg/kg
Cation exchange Capacity	28	cmol/kg
Sulphate – S	42	mg/kg
Chloride – Cl	31	mg/kg
Boron	0.58	mg/kg
Zinc (DTPA)	0.32	mg/kg
Copper (DTPA)	0.62	mg/kg
Iron (DTPA)	2.42	mg/kg
Manganese (DTPA)	0.68	mg/kg
K Cation	3.93	%
Ca Cation	86	%
Mg Cation	9	%
ESP	0.74	%

Element	Result	Units
K:Mg	0.44	Ratio
Ca:Mg	9.6	Ratio
C:N	0.08	
Soluble Silica	52	Ppm

Comments on soil results

The alkaline nature of soil in this sample is typical of the region. The soil has high levels of underlying limestone of variable particle size. Soil phosphorus levels are low and potassium levels are high which is typical of most soils in this region. The hot dry conditions and low rainfall results in very low levels of organic carbon.

The soluble silica was measured using the calcium chloride extraction method and found to be 52ppm suggesting that this soil type would be responsive to applications of silica.

The property is devoted to the production of organic dates so the lower nutrient levels in comparison to more intensive systems in line with soil nutrient levels in this region. The regional clay loam based soils are traditionally high in potassium and trace element levels (low) are normal for this soil type.

Plant phenology data

Fronds and leaflets were measured from the youngest mature frond to determine any potential variance between treatments. Measurements were taken twice over the growing season with individual data collated and compared between and within treatments for analysis of variance.

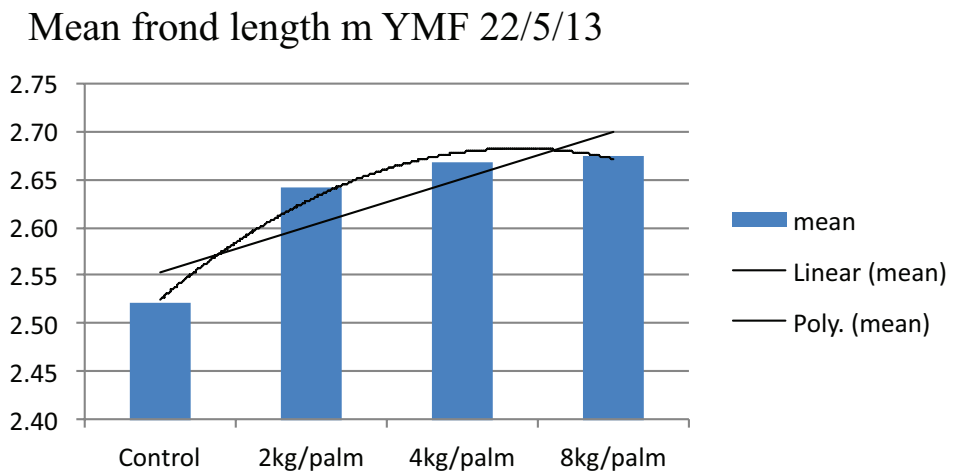
Table 3: Frond length sample dates 22/5/13 and 18/10/13 - **22/5/13**

	Control	2kg/palm	4kg/palm	8kg/palm
	2.52	2.61	2.71	2.68
	2.53	2.63	2.69	2.63
	2.53	2.59	2.69	2.85
	2.49	2.6	2.79	2.75
	2.53	2.6	2.67	2.63
	2.49	2.62	2.75	2.64
	2.53	2.67	2.61	2.7
	2.63	2.89	2.61	2.6
	2.51	2.52	2.67	2.59
	2.49	2.65	2.7	2.86
	2.52	2.64	2.47	2.81
	2.57	2.63	2.47	2.51
	2.5	2.68	2.63	2.53
	2.52		2.88	
	2.46			
	Control	**2kg/palm**	**4kg/palm**	**8kg/palm**
Mean	2.52	2.64	2.67	2.68
STDEV	0.04	0.08	0.11	0.11
Average deviation	0.02	0.05	0.07	0.09

18/10/13

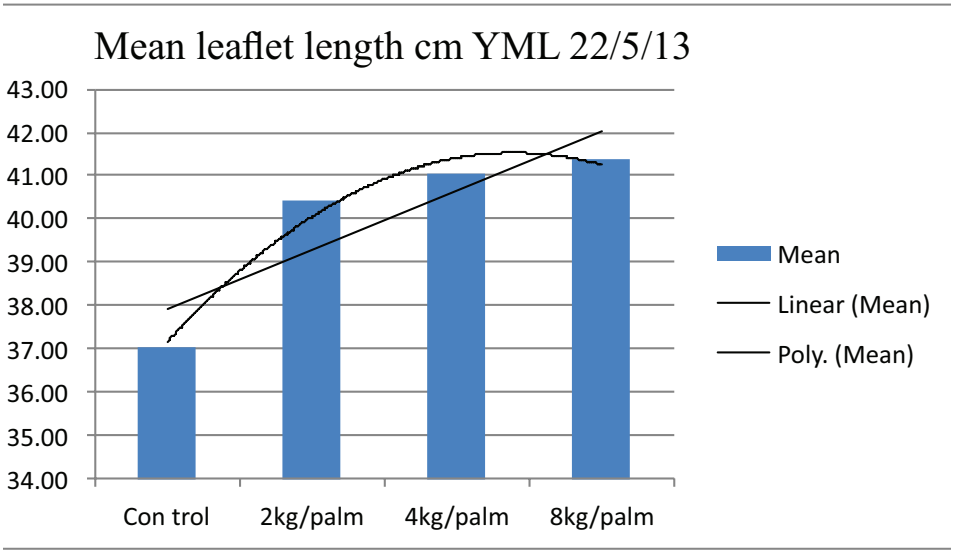
	Control	2kg/palm	4kg/palm	8kg/palm
	2.78	2.65	2.9	2.78
	2.51	2.75	2.84	2.78
	2.47	2.67	2.69	2.7
	2.6	2.78	2.64	2.56
	2.47	2.7	2.7	2.7
	2.36	2.71	2.82	2.86
	2.47	2.67	2.63	2.68
	2.6	2.67	2.75	2.66
	2.42	2.8	2.75	2.55
	2.39	2.66	2.75	2.74
	2.39	2.57	2.56	2.82
	2.43	2.55	2.57	2.64
	2.42	2.68	2.71	2.53
	Control	**2kg/palm**	**4kg/palm**	**8kg/palm**
Mean	2.49	2.68	2.72	2.69
STDEV	0.12	0.07	0.10	0.10
Average deviation	0.02	0.05	0.07	0.08

The tabled data above highlights quite considerable differences in mean frond length between the control and rows where Agripower Si has been applied. The increase in frond length in the treated palms was maintained throughout the current growing season and that untreated palms did not recover in average frond growth rates over the current growing season. The graphs below represent this data in a more visual format.



Graph 1: Mean frond length youngest mature frond (YMF) in metres 22/5/13

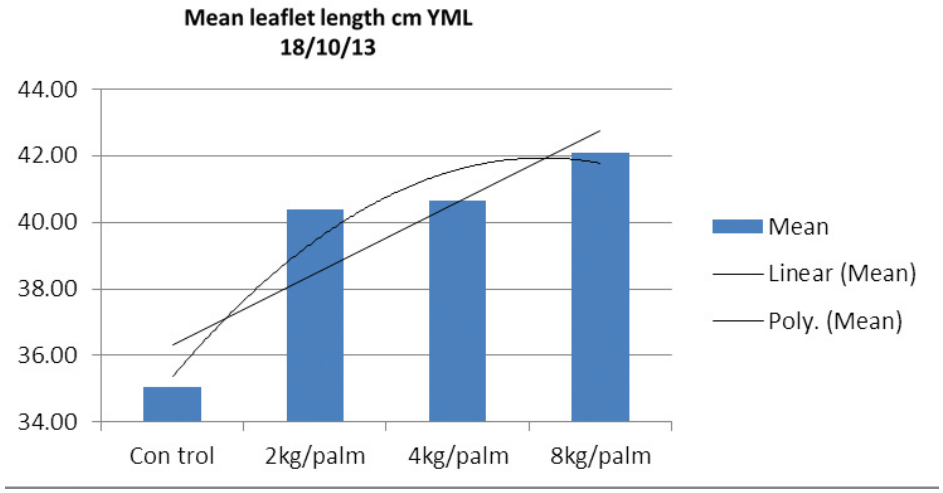
Mean frond length can be seen to be greater in the treated over the untreated palms within the same planted area in the trial block. By applying a polynomial trend line to the data the aim was to determine potential limiting rates to Si application as influencing frond length.



Graph 2: Mean leaflet length from the mid leaflet of the youngest mature leaflet (YML). 22/5/13

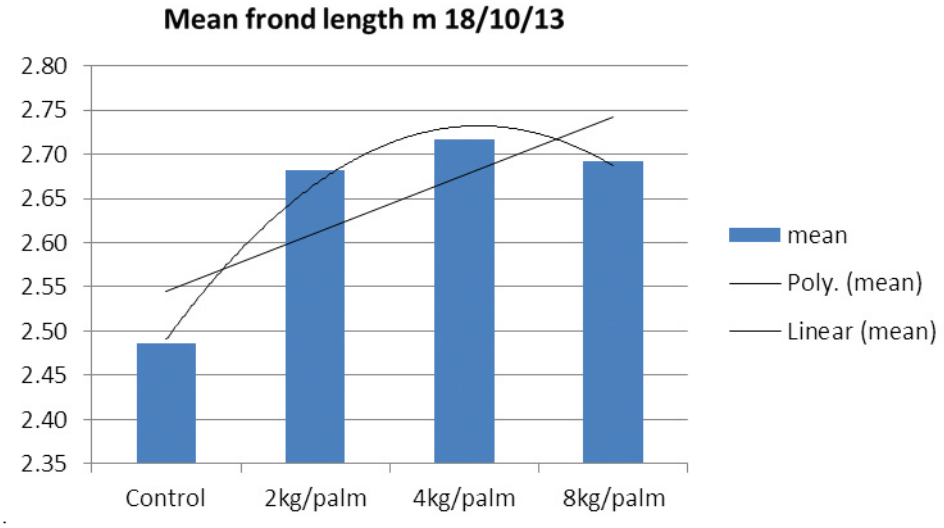
Leaflet length and frond length are in line with each other and would suggest that leaflet length correlated to frond length in a date palm.

Further frond and leaflet samples were taken on the 18/10/13. The aim of these measurements was to see if any correction or changes to leaflet and frond length had occurred in the 5 months from the original sampling date.



Graph 3: Mean Leaflet Length 18/10/13

Data from the 18/5/13 is in line with sample data from the 22/5/13. There has been minimal change to leaflet length from the mid part of the frond since the original sampling.



Graph 4: Mean frond length 18/10/13

Data from the 2 sampling dates indicate that growth response to applications of the Agricpower Si product occur early in the development phase of the frond and leaflets and that this response is held over time in the plant. This would suggest that the impact of the vegetative response is made earlier in the growth phase and that relatively small applications of Agripower Si have resulted in quite significant increases in vegetative growth.

Tissue test results

Tissue analysis was conducted on both the 14/11/12 and 20/9/13 to determine if there were any significant differences in plant nutrient levels between treated and untreated palms. Leaflets were taken from each treated palm to form a composite tissue sample for analysis.

Table 4: Dried tissues analysis 14/11/12

Nutrient	Control	2kg Palm	4kg Palm	8 Kg/Palm
B ppm	16	15	15	14
Ca %	0.27	0.27	0.21	0.21
Cu ppm	118	108	89.5	61.8
Fe ppm	55	63	50	50
K %	2.02	2.02	2.06	1.91
Mg %	0.21	0.22	0.21	0.2
Mn ppm	25	26	23	23
Mo ppm	0.01	0.01	0.01	0.01
N %	1.62	1.49	1.4	1.46
NO3-N ppm	7	9	4	9
Na %	0.05	0.05	0.05	0.05
P %	0.14	0.14	0.16	0.14

Nutrient	Control	2kg Palm	4kg Palm	8 Kg/Palm
S %	0.15	0.16	0.14	0.14
Si mg/kg	1387	689	698	692
Zn ppm	15	15	15	14

Sample Date 20/9/13

Nutrient	Control	2kg Palm	4kg Palm	8 Kg/Palm
B ppm	42	37	46	43
Ca %	1.01	0.95	1.08	0.99
Cu ppm	13.4	13.7	17.6	14.9
Fe ppm	70	70	88	69
K %	0.57	0.73	0.59	0.63
Mg %	0.39	0.37	0.4	0.37
Mn ppm	40	43	46	51
Mo ppm	0.39	0.40	0.36	0.38
N %	1.72	1.73	1.75	1.77
NO3-N ppm	20	21	18	18
Na %	0.05	0.05	0.05	0.05
P %	0.15	0.16	0.15	0.15
S %	0.25	0.16	0.24	0.27
Si mg/kg	3374	3015	3045	2642
Zn ppm	18	17	15	15

The only consistent data in both sets of tissue data is that the more Si that has been added to the plant, the lower the amount in leaflet tissue analysis. It is assumed that the reason for this variance is due to a dilution factor in the tissue as a function of increased vegetative growth. It is also possible due the physiology of the date palm other parts of the plant may be a major sink for Si and that tissue analysis while reflecting Si (and other nutrient levels at a point in time), do not reflect total nutrient distribution within the plant.

From observation of the data, Date Palms can be seen to have a high leaflet content of Si to the extent that this nutrient could be regarded as one of the major plant nutrients for this species. These are in line with the observations made by Matichenkov and Bocharnikova (2006).

SUMMARY AND CONCLUSION

A field level study into plant nutrition effects is not an exact science since it is difficult to achieve perfect replicas of the same plant for the study in a field situation. Small variations in soil type and hydraulic distribution of water within the root zone can have significant physical effects on plant growth as well as genetic variance between plants of the same variety. However in spite of this natural variance applications of Silica have resulted in visually significant and measurable changes in plant growth and development. This is in line with published data showing the significance of silica on the growth and development of date palms in other parts of the world.

While the length of this research has not been able to quantify the impact of Silica on fruit loads in date palms in terms of increased water use efficiency and drought tolerance, the increased growth achieved through applications of

Agripower Si in vegetative mass is encouraging.

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Effects of arbuscular mycorrhizal fungi on growth and physiology of date palm seedling under phosphorus deficit

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ABSTRACT

Arbuscular mycorrhizal fungi are endophytic fungi that enhance plant growth and biomass production in arid and sub arid areas where phosphorus is massively under insoluble forms in soil. Earlier works established that the enhanced biomass results from improved nutrient status. In particular, Arbuscular mycorrhizal fungi mediate phosphorous supply. The objective of this study is to evaluate the role of arbuscular mycorrhizal fungi (Glomus manihotis) in solubilizing rock phosphate enhancing thereby growth of date palm (*Phoenix dactylifera* L.) seedlings under phosphorus deficit. Young germinations of date palm were grown on inert substrate containing 5g of rock phosphate and inoculated or not with Glomus manihotis. Cultures were irrigated with Hoagland solution containing or not KH_2PO_4 as phosphorus source. After two months, growth, physiological and biochemical parameters were assessed in mycorrhizal (AM-plants) and non mycorrhizal plants (Non-AM). Obtained results showed that mycorrhizal colonization induces an increase in growth parameters (fresh weight and plant height) and biomass production (shoot and roots dry weights) regardless phosphorus treatment. In the presence of rock phosphate as the only source of phosphorus, mycorrhizal date palm seedlings showed increased acid phosphatase, activity as well as higher level of soluble sugar, higher relative water content and higher stomatal conductance compared to non-inoculated plants. However, the guaiacol peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings under phosphorus deficit.

Keywords: date palm seedlings, arbuscular mycorrhizal fungi, Phosphorus deficiency, rock phosphate solubilization.

1. INTRODUCTION

Plants of arid and semi-arid areas including date palm are often faced with the combined effect of several biotic and abiotic stresses. In addition to the lack of water, which is the main limiting factor of growth, soils are generally poor in essential nutrients such as phosphorus (Diem *et al.,* 1981; Mikola, 1987). Under such difficult environmental conditions, the quality of the root system and the efficiency of its association with the soil microorganisms may play an important role in plant development. In these extreme conditions, plants survival is likely due to the result of the symbiotic association between root and soil borne arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal symbiosis is known to benefit mineral nutrition and to provide improved water relations thereby enhancing host plant protection against the detrimental effects of environmental constraints such as salinity (Klironomos *et al.,* 2001; Giri *et al.,* 2003), water and nutrients deficiency

(Aqqua *et al.,* 2010; Faghire *et al.,* 2010 ; Baslam *et al.,* 2014) and resistance to pathogenic soil microorganisms (Garmendia *et al.,* 2004). The present work aims to evaluate the effect of arbuscular mycorrhizal fungi on growth and physiology of date palm seedlings under phosphorus deficit.

2. MATERIAL AND METHODS

Seeds of date Palm were disinfected with sodium hypochlorite (20%) for 20 minutes and germinated in sterile wet sand at 38°C in the dark. Two weeks later, germinating seeds were transplanted in pot containing 1kg of inert substrate containing or not 5 g of rock phosphate and inoculated or not with 5 g of inoculum of *Glomus manihotis* consisting of a soil mixture of spores and mycorrhizal roots fragments of barley (Meddich et al. 2000). Cultures were then irrigated with Hoagland solution containing or not KH_2PO_4 as phosphorus source. After four months date palm plantlets were harvested and growth parameters (shoot height and shoot fresh weight) as well as biomass production (shoot and roots dry weights) were measured. Plantlets water status was examined through the evaluation of the relative water content (RWC) and stomatal conductance. Measurements of the stomatal conductance were carried out during a sunny day on the leaf surface of a single leaf per plant using a diffusion porometer SC-1.

Biochemical analysis including enzymes essays were carried out on crude extract obtained from 0.1g of date palm leaves that were ground with 0.02g of polyvinylpolypirrolidine (PVPP) in a cold mortar and soaked with 2 ml of phosphate buffer 0.1 M (pH 7.0) containing 0.0168g $EDTANa_2$ and 0.1 g of polyvinylpolypirrolidine (PVPP) at 1%. The mixture was then centrifuged (15 000xg) for 15 min at 4 °C and the supernatant recovered was stored at 4 °C until analysis of the various enzymatic activities. Acid phosphatase activity was determined by measuring the amount of para-nitrophenol (pNP) released by using a UV-visible spectrophotometer at 405 nm (Araùjo *et al.,* 2008). Activity of guaiacol peroxidase (G-POX) was determined according to the method of Putter (1974) by following the increase of the absorbance caused by the appearance in the medium of oxidized guaiacol. Polyphenol oxidase as (PPO) activity was determined in a reaction mixture containing 200 µL of phosphate buffer (0.1 M), 500 µL of catechol (10 mM), 100 µL of enzyme extract and 250 mL of H_2O_2. After 3 min of incubation at ambient temperature, the absorbance was determined at 410 nm. Total soluble sugars were determined by the method of Yemm et Willis (1954). It is to take 50µL of plant extract, 450µL of phosphate buffer and 3 mL of anthrone reagent in clean glass tubes. The tubes are placed in a water bath at 100 ° C for 15 min. Reading the absorbance at 620 nm is performed. The values obtained are reported in the standard range.

Statistical analysis

The results were statistically analyzed using SPSS software. This analysis includes an ANOVA 2 followed by means comparison using LSD test at 5%.

RESULTS

3.1. Growth parameters

Plant growth parameters varied significantly depending on the phosphorus treatment and mycorrhizal inoculation (Table 1). Plan height and shoot and root fresh weights were greatly reduced in plantlets irrigated with nutrient solution without soluble phosphorus compared to those irrigated with complete nutrient solution regardless mycorrhizal inoculation. This reduction was more pronounced in non-inoculated than in mycorrhizal plants. In the absence of soluble P, plants cultivated on substrate added with rock phosphate showed higher plant growth parameters than those cultivated without any source of P. Moreover, in the presence of rock phosphate as the only source of P, mycorrhizal plants grow higher compared to the respective non-mycorrhizal plants and to the plants irrigated with nutrient solution without P. The lack of any source of P generates a significant reduction in biomass production in both mycorrhizal and non-mycorrhizal plants (Table 1). In mycorrhizal plants, the addition of rock phosphate allows a more relevant increase of root and shoots dry weights than in non-mycorrhizal plants. Non-inoculated plants and plants cultivated without any source of phosphorus produce less dry material (Table 1).

3.2. Physiological parameters

Values of relative water contents vary significantly regarding phosphorus treatments (Table 2). Water accumulated by the leaves of date palm seedlings remains increasingly important in the presence of phosphorus. Mycorrhizal plants showed the highest values of RWC regardless phosphorus status.

Stomatal conductance varies significantly with phosphorus treatment and mycorrhizal status (Table 2). Under phosphorus deficiency mycorrhizal plants recorded higher stomatal conductance value compared to non-inoculated plants (Table 2).

Activity of acid phosphatase was lower in plants treated with complete nutrient solution. The highest acid phosphatase activity was recorded in mycorrhizal plants with the rock phosphate (RP) as the only source of phosphorus. However, the guaiacol peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings regardless phosphorus treatments. The peroxidase and polyphenoloxidase activities were increased by P deficit regardless mycorrhizal status. Under phosphorus

deficit soluble sugar content was lower in leaves of non-inoculated plants than in mycorrhizal ones (Table 2).

DISCUSSION

This study investigated the influence of arbuscular mycorrhizae on the growth and physiology of date palm seedlings under phosphorus deficit. The results showed that plants growth and biomass production varied significantly with the applied phosphorus treatments. The highest values were recorded in AM-plant compared to NM-plant under all phosphorus treatments. The same results were observed by Bowen et Théodorou (1967) who showed an increase in dry material of *Pinus radiata* seedlings inoculated under phosphorus fertilization compared to non-inoculated seedlings. This increase was related to the role of arbuscular mycorrhizal fungi in enhancing uptake of $H_2PO_4^-$ (Gillespie and Pope, 1991).

On the other hand, values of the stomatal conductance vary differently according to mycorrhizal status and phosphorus treatments. Under phosphorus deficit stomatal conductance was highest in mycorrhizal seedlings. Similar results were reported by Augé et al (1986) showing low osmoticums in non-mycorrhizal plants under phosphorus deficiency. The enhanced stomatal conductance go along with increased levels of relative water content (Allen and Boosalis, 1983 ; Bildusas et al, 1986) as well as increased soluble sugar concentration (Suresh and Bagyaraj 1984).

Activity of acid phosphatase was lowest in plants treated with complete nutrient solution and highest in mycorrhizal plants with RP as the only source of P. This was probably due to the lack of soluble phosphorus in the soil and the availability of other forms of insoluble phosphorus (RP) which requires high activity of acid phosphatase for its solubilization (Mousain et Salsac 1986). The peroxidase and polyphenoloxidase activities were increased by P deficit regardless mycorrhizal status. Peroxidase and polyphenoloxidase activities were highest in non-inoculated seedlings. Similar result were reported by Avdiushko et al (1993) and Zheng et al (2005), suggesting the contribution of these enzymes in the catalysis of the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure.

CONCLUSION

Phosphorus deficiency is one of the major problems in palm grove ecosystems that affect plants growth and productivity. However, this investigation showed that arbuscular mycorrhizal colonization can improve date palm growth and biomass production under phosphorus deficit through, 1) enhancing plants water status, 2) increasing phosphorus availability by enhancing acid phosphatae activity that contributes to phosphorus solubility, and 3) the activation of enzymes catalyzing the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure.

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Tables

Table 1 : Effect of arbuscular mycorrhizal fungi on growth and biomass production in date palm seedlings under phosphorus deficit

P Treatment	Mycorrhizal status	% M	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Complete nutrient solution	NM	0	30,1bc	19,3b	15,5b	12,4ab	12,0bc
	AM	63.3b	35,8a	22,8a	17,7a	14,1a	14,5a
Nutrient solution without P + Rock P	NM	0	28,8bc	14,9cd	14,7bc	10,9cd	11cd
	AM	69a	31,9ab	15,6c	15,9b	11,8bc	12,4b
Nutrient solution without P	NM	0	26,0c	12,7d	12,7d	9,5d	9,6e
	AM	60c	28,4bc	13,3cd	13,7cd	10,1cd	10,1de

Table 2: Effect of arbuscular mycorrhizal fungi on physiology of date palm seedlings under phosphorus deficit

P treatments	Mycorrhizal status	Stomatal conductance (µmol/m2)	Relative water content (%)	Acid phosphatase activity (UE/ mg of protein)	Guaiacol Peroxidase activity (UE/ mg of protein)	Polyphenol activity (UE/ mg of protein)	Soluble sugar (µg/ mg of fresh material
Complete nutrient solution	NM	115,47e	64,44a	0,47e	77,63c	57,29f	62,30bc
	AM	161,27c	82,97ab	0,27d	36,81ab	37,72e	67,39ab
Nutrient solution without P + Rock P	NM	125,03de	55,62b	0,68c	102,93a	108,50b	58,62c
	AM	216,03b	60,98ab	2,79a	54,21bc	67,37d	71,15a
Nutrient solution without P	NM	136,00d	46,70b	0,41d	106,58a	168,90a	30,91e
	AM	233,67a	50,66b	1,85b	74,37ab	77,62c	45,22d

Arbuscular mycorrhizal fungi enhanced date palm tolerance to water deficit

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) has a high hardiness and adaptability to the climatic conditions of the arid and sub-arid areas where it may represent not only a biological tool to counteract processes of erosion and desertification, but also provide adequate microclimate for the establishment of other species, playing thereby the role of pioneer and productive species. However, low water availability and nutrients deficit exacerbated by the effects of climate fluctuations that are particularly marked in these areas severely limit agricultural production. During the last decades, the management of the arbuscular mycorrhizal (AM) fungi as providers of key ecological services has been at the forefront of generating and promoting agricultural production technologies. These soil microorganisms can form a symbiotic association with roots of most land plants and can participate in improving plant growth and nutrition, strengthening plant performance, restoring ecosystems and combating pests and pollution. Several studies have clearly highlighted the fundamental role that mycorrhizal fungi play at the interface between the soil and plant roots enhancing thereby the multitrophic and protective interactions that affect productivity, competitiveness and survival of the majority of plant species both in natural ecosystems and in managed field. In this presentation, we will discuss results of our recent investigations showing the positive effects of arbuscular mycorhizal symbiosis on date palm tolerance to water. We will discuss the most persuasive and effective use of these fungi as biofertilizers-biostimulative to improve date palm growth and physiology under low water availability.

Keywords: Sustainable agriculture, Mycorrhizal symbiosis, Arbuscular mycorrhizae,

INTRODUCTION

In last decades, the oasis ecosystem was subjected to different environmental constraint such as poor soils, water scarcity, sand invasion and desertification causing considerable economic, ecological and social damage. The recent worsening of these problems is the result of the detrimental effects of the global climate changes that are more pronounced in these arid and semi-arid areas. These constraints cause not only reduction in the production of dates, the principal food of humans and animals in the desert, but also an imbalance of the oasis ecosystem causing a serious threat to the plant resources and the long-term agricultural production in these difficult environments (Haddouch, 1997). Arbuscular mycorrhizas are highly evolved mutualistic associations formed between soil-borne fungi and plant roots. AM symbiosis is known to benefit mineral nutrition and to provide enhanced water relations thereby enhancing host plant protection against the detrimental effects of environmental constraints (Stutz *et al.,* 2000). In exchange, the plants supply mycorrhizal fungi with carbon fixed using photosynthetic process. The management of mycorrhizal fungi as providers of key ecological benefits has been at the forefront of generating and promoting agricultural production technologies. These microorganisms often referred to as “ecosystem engineers”, can participate in improving plant growth and nutrition, strengthening plant performance, restoring ecosystems and

combating pests and pollution. In difficult areas AMF may represent not only a biological mean to counteract processes of soil degradation, but also a challenge for the development of the long-term agricultural production. The purpose of this paper is to review some of our recent research results that deal with the effect of AM symbiosis on promoting date palm tolerance to water deficit. We will discuss the most persuasive and effective effect of these fungi in improving date palm growth and physiology under low water availability.

MATERIALS AND METHODS

Seeds collected from the date palm population of the palm grove of Marrakech were surface-sterilized by immersing in 70 % alcohol for 5 min, rinsed three times with distilled water and germinated on wet paper in pans at 38°C. After two weeks seedlings were transplanted into plastic pots containing 1kg of autoclaved soil collected from the palm grove of Marrakesh. The experimental pots were placed in greenhouse under natural light condition. Mycorrhizal inoculum from our own stock culture consisted of soil spores and hyphae and infected root fragments from rhizospheric soil of mycotrophic plant. Ten grams of inoculum was used per pot and placed 5 cm below date palm roots. Non-mycorrhized seedlings received the same weight of autoclaved inoculum.

Water treatments began 4 months after AM inoculation. Well watered pots (WW) were watered with 75% of field capacity and water stressed (WS) pots received 25% of field capacity. The water status of the pots was daily examined and the amount of water loosed was refilled into each pot. After 8 weeks of water treatments, plants were harvested and growth parameters (plant height and root length) and biomass production (shoot and root dry weights) were recorded. Water relation parameters were measured as previously described (Faghire *et al.,* 2010). Relative water content (RWC) was calculated using the technique described by Turner (1981). Leaf water potential (Ψw) was measured by the method of chamber pressure developed by Scholander et al. (1965). Osmotic potential at full turgor ($\Psi\pi 100$), osmotic potential at turgor loss ($\Psi\pi 0$), symplastic water (WS), and cell elasticity modulus (ξ) were obtained from the pressure-volume curve method (Tyree and Hammel, 1972).

Biochemical changes including superoxide dismutase (Beyer & Fridovich, 1987), catalase (Aebi, 1984), guaiacol peroxidase (Polle *et al.,* 1994) and ascorbate peroxidase (Amako *et al.,* 1994) activities were determined. Proline (Paquin and Lechasseur 1979), soluble protein (Bradford (1976) and total soluble sugar (Yemm and Willis 1954) contents were estimated as described by balsam *et al.,* (2009). Leaf antioxidant enzyme activities SOD, CAT, APX and GPOX were determined. Malondialdehyde was measured by the thiobarbituric acid method as described by Heat and Packer (1981) and H_2O_2 by using titanium method according to Patterson and al. (1984).

The experimental treatments consisted of two watering regimes (well watered and water stress) and were arranged in a complete randomized block design. Each treatment was replicated ten times. 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 AND DISCUSSION

AM symbiosis increased growth and biomass production of date palm seedlings under both well watering and water restricted conditions. Plant height, root length and shoot and root dry weights were more sensitive to mycorrhizal colonization under water limiting regime. Improved growth under water stress is due to the action of AM fungi on 1) the improvement of water and nutrients absorption, 2) the maintenance of water relations such as increased relative water content and symplastic water content, maintained water potential and osmotic potential and the cell turgor, 3) the induction of osmotic adjustment through the accumulation of organic osmolytes such as sugar and proline and inorganic osmolytes such as K+, and 4) the protection of the plants against oxidative stress by reducing ROS production and increasing antioxidant enzymes activities responsible for the elimination of the ROS.

Our results showed that arbuscular mycorrhizal fungi had positive effects on nutrient concentrations analyzed in the tissues of date palm plants under water stress. Indeed tissue nutrients (P, K, Ca and Mg) concentrations were higher in mycorrhizal plants (AM-P) than in non-inoculated (N-AM) ones. Water stress induced a significant decrease of plant nutrient concentrations (Table 1). This negative effect was more pronounced in N-AM than in AM-P. These Results were in accord with the finding that has been reported for date palm (Oihabi, 1991; Al-Whaibi and Khaliel, 1994; Meddich *et al.,* 2004; Faghire et al. 2010) and other plant species (Ruiz-Lozano and Azcon, 1996; Wu and Xia, 2005). AMF have been shown to improve productivity in soils of low fertility and are particularly important for increasing the uptake of slowly diffusing ions such as PO_4^{3-}, immobile nutrients such as P, Zn and Cu, and other nutrients such as ammonium and potassium (Rhodes and Gerdemann, 1980; Liu *et al.,* 2002). Under drought conditions the uptake of highly mobile nutrients such as NO_3^- can also be enhanced by mycorrhizal associations (Subramanian and Charest, 1999). The most established benefits from mycorrhizal fungus to the host plant is through the widespread mycelial network which penetrates deeper and wider in the soil in search of water and nutrients thereby widening the zone of activity. Nutrient acquisition begins with the uptake of free nutrients

from soil by fungal extra-radical hyphae that act as a bridge between the soil and plant roots (Bucher, 2007). Nutrients are then transferred through the periarbuscular membrane to the plant cytosol. The majority of this nutrients exchange is believed to occur within root cortical cells containing highly-branched hyphal structures termed arbuscules. The establishment of the mycorrhizal network offers a number of basic advantages for the acquisition of mineral nutrients: 1) fungal hyphae extend beyond the nutrient depletion zone that develops around the root. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion. AMF hyphae can readily bridge this depletion zone and grow into soil with an adequate supply of nutrients. 2) Fungal hyphae network greatly increase the surface area for the absorption of nutrients relative to non-mycorrhizal roots. 3) Due to their narrow diameter relative to roots, hyphae are able to extend into soil pores that are inaccessible to roots or even root hairs, 4) Mycorrhizal fungi can access forms of N and P that are unavailable to non-mycorrhizal plants, particularly organic forms of these nutrients. One mechanism for this access is the production by plant roots and the associated mycorrhizal fungi of organic acids and hydrolytic enzymes.

Parameters related to water status (Table 2) showed that water stressed mycorrhizal date palm seedlings maintained better water relations in terms of relative water content, water potential and turgid potential compared to non-inoculated seedlings. 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 favorable plant water relations is vital for the development of drought adaptation in crop plants (Auge RM, 2001). The higher RWC and Ψw and the lower WS 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 Ψπ 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.,* 2004). Mycorrhizal associations improve water uptake by increasing the hydraulic conductivity of the roots either by modifying root morphology and root anatomy or indirectly by hormonal and structural changes in the host plant. The survival of mycorrhizal plants in extremely dry condition is the result of a better root performance and the ability to explore water in wider zones of soil by extension of the fungal mycelium into non-rhizospheric soil (Kehri and Chandra, 1990).

Biochemical analysis emphasized the role of AMF in plants water stress tolerance by increasing antioxidant enzymes (SOD, P-POD, APX and PPO) activities and sugars and protein and accumulation and reducing MDA and H2O2 accumulation (table 3, 4). Compared to non-inoculated plants, mycorrhizal plants subjected to water stress accumulated more proteins, showed high level of soluble sugars and low accumulation of malonyldialdehyde and hydrogen peroxide. Similarly, mycorrhizal plants showed an increase in the activities of superoxide dismutase (SOD) and ascorbate and guaiacol peroxidase and polyphenol oxidase (Baslam et al. 2010; Faghire et al 2010; 2013; Fouad et al. 2012, 2013). In addition, oxidative damage, estimated as the ratio of malondyaldehyde / protein was higher in non inoculated seedlings and lowest in mycorrhizal seedlings (Baslam et al. 2010; Faghire et al 2010; 2013; Fouad et al. 2012, 2013). Our results suggest that the increased activity of antioxidant enzymes and decreased concentration of ROS compounds found in AM plants may serve to protect them against oxidative damage, enhancing thereby plants tolerance to water stress.

CONCLUSION

Contribution of the AM symbiosis to date palm drought tolerance is the result of their action on several plant functions including nutritional, physiological and biochemical processes. This appears to be due in many instances to differences in tissue hydration between AM-P and N-AM plants. However, AM symbiosis enhance drought tolerance of host plants through many mechanisms, such as increased water and nutrients absorption, high stomatal regulation by hormonal signals, enhanced osmotic adjustment, higher root hydraulic conductivity and leaf hydration, and reduced oxidative damage caused by the reactive oxygen species (ROS) generated during drought. A greater osmotic adjustment has also been reported in leaves of mycorrhizal plants than in non-mycorrhizal ones during drought period. In the same way, AM plants had postponed declines in leaf water potential during drought stress. Farther more, mycorrhizal plants have operated special biochemical mechanisms that prevent plant cell from oxidative damage through accumulation of some antioxidants compounds and enhancing antioxidant enzymes activities in leaves and roots of water stressed plants, and this was correlated to plant protection against drought.

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Tables

Table 1: Mycorrhizal colonization, Plant height, Root length, shoot dry weight and root dry weight and nutrients contents of non-mycorrhizal (Non-AM) or mycorrhizal date palm seedlings grown under well watered (WW) or water stress (WS) conditions.

Water régime	AMF status	M (%)	SDW (g)	RDW (g)	SH (cm)	RL (cm)	P (mg)	Ca (mg)	Mg (mg)	K (mg)
WW	N-AM	0	3,16b	1,76b	22,5c	27,9c	1,69b	0,5b	0,72b	1,36c
	AM-P	61,4a	6,41a	3,38a	44,7a	60,5a	6,08a	0,88a	1,94a	2,44a
WS	N-AM	0	1,43c	0,81c	13,3d	18,9d	1,62b	0,23c	0,39b	0,69d
	AM-P	42,6b	3,21b	2,97a	26,6b	46,9b	5,78a	0,60b	2,16a	1,97b

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2: Relative water content (RWC), Leaf water potential (Ψ_w), Symplastic water (W_S), Osmotic potential at full turgor ($\Psi\pi^{100}$), Osmotic potential at turgor loss ($\Psi\pi_0$) and cell Modulus of Elasticity (ξ) of mycorrhized (AM) and non-mycorrhized (Non-AM) date palm seedlings subjected to two watering treatments.

Water regime	AMF Status	RWC (%)	Ψ_w (Mpa)	W_S	$\Psi\pi^{100}$ (Mpa)	$\Psi\pi^0$ (Mpa)	ξ
WW	N-AM	98.62 a	-30.5 a	5.6 a	-4.8 b	-15.3 b	1.77 b
	AM-P	99.11 a	-27.2 b	5.3 b	-6.9 a	-25.0 a	3.52 a
WS	N-AM	93.48 b	-33.6 b	2.5 a	-20.0 a	-28.6 a	1.32 a
	AM-P	96.96 a	-37.0 a	1.7 b	-13.3 b	-25.0 b	0.54 b

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 3: Malonyldialdehyde (MDA), hydrogen peroxide (H_2O_2), soluble sugar and proline contents and oxidative damage in leaves of non-mycorrhizal or mycorrhizal date palm seedlings grown under well water or water stress conditions.

Water régime	AMF status	MDA (nmol.g^{-1} DM)	H_2O_2 (μmol.g^{-1} DM)	TSS (mg.g^{-1} DM)	Proline (nmol.g^{-1} DM)	OD (nmol MDA mg^{-1} prot)
WW	N-AM	53.2b	24.1b	54.6c	4105.2c	12.42b
	AM-P	38.6d	24.2b	78.7a	9193.2a	6.88c

Water régime	AMF status	MDA (nmol.g^{-1} DM)	H_2O_2 (µmol.g^{-1} DM)	TSS (mg.g^{-1} DM)	Proline (nmol.g^{-1} DM)	OD (nmol MDA mg^{-1} prot)
WS	N-AM	65.5a	28.4a	47.5d	3570.2d	20.47a
	AM-P	42.1c	27.3a	68.5b	4070.4c	6.35c

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 4: Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (G-POD) activities in leaves of non-mycorrhizal or mycorrhizal date palm seedlings grown under well water or water stress conditions.

Water statut	AMF statut	SOD (USOD g^{-1} DM min^{-1})	G-POD (mmol.g^{-1} DM min^{-1})	CAT (nmol g^{-1} DM min^{-1})	APX (mmol g^{-1} DM min^{-1})
WW	N-AM	652,3a	3,07c	116,7cd	2,11d
	AM-P	447,7c	4,20b	136,8c	2,89d
WS	N-AM	596,1b	4,44b	225,5a	4,68c
	AM-P	667,4a	5,87a	104,41d	7,05a

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Mycorrhizal symbiosis alleviated reactive oxygen species accumulation in date palm under water deficit

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ABSTRACT

The scarcity of water is producing a generalization of drought effects in most of plant species in arid and sub-arid areas. Reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{1-}) are generated in plants undergoing water deficit and can cause oxidative damage to lipids, proteins and nucleic acids leading to loss of membrane integrity and cell death. The induction of ROS-scavenging enzymes, such as SOD, CAT, APX and GPX is the most common mechanism for detoxifying plant tissue. The present study was focused on determining the effect of arbuscular mycorrhizal AMF on antioxidant enzymes activites and non-enzymatic antioxidants production in leaves of date palm seedlings subjected to well-watered (75% of field capacity) and water stressed (25% of field capacity) conditions. Obtained results showed that water stress steeply decreased plant growth (shoot height) and biomass production (shoot and root dry weights) in non-inoculated date palm seedling compared to AM-plants. Leaves of water stressed mycorrhizal plants showed lower malondialdehyde (MDA) and H2O2 and O2 concentrations than their relative non-inoculated plants. Moreover, mycorrhizal symbiosis notably increased the activities of guaiacol peroxidase (G-POD) and ascorbate peroxidase (APX) under water stress. Soluble sugar and prolin contents were also enhanced in leaves of mycorrhizal plants subjected to water stress. Our results suggest that the increased concentrations of antioxidant enzymes activities and non-enzymatic antioxidants contents found in mycorrhizal plants may enhance date palm protection against oxidative damage enhancing thereby drought tolerance.

Key words: Arbuscular mycorrhizal fungi, date palm, drought, antioxidant metabolism, oxidative damage.

INTRODUCTION

The lack of adequate soil moisture leading to low water availability is the most common characteristic in arid and semi-arid regions. The water scarcity produces a generalization of water stress effects in most plant species in arid land. Water stress is often associated with increased levels of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{1-}) which are generated in most plants undergoing water deficit and can seriously destroy the normal metabolism of the plant causing oxidative damage to lipids, proteins and nucleic acids leading to loss of membrane integrity and cell death. To maintain growth and productivity plants have to prevent accumulation of these harmful species as rapidly as possible. The induction of ROS-scavenging enzymes, such as SOD, CAT, APX and GPX is the most common mechanism for detoxifying plant tissue.

It well established that Arbuscular mycorrhizal fungi (AMF) can form symbiotic association with the vast majority of land plants including those of the arid areas. AMF play a critical role in plants mineral nutrition and terrestrial

ecosystem functioning. Once established, AM association benefits host plants not only by improving water uptake and mineral nutrition (Aqqua et al. 2010), but also by increasing plant resistance to drought (Faghire *et al.,* 2010; Baslam *et al.,* 2013), soil salinity (Giri *et al.,* 2003) and pathogens (Garmendia *et al.,* 2004). In date palm, AM symbiosis was recognized as positively significant for growth, nutrients and water status, and plantlets establishment and survival especially on poor soil (Oiahabi 1991; Meddich, 2000; Baslam *et al.,* 2008, 2009; Aqqua *et al.,* 2010; Faghire *et al.,* 2010; Baslam *et al.,* 2013). In earlier studies we have shown that AM fungi allow for greater uptake of nutrients and play an important role in improving water relations thereby enhancing date palm growth under water deficiency (Faghire *et al.,* 2010; Aqqua *et al.,* 2010; Baslam and al., 2008; 2009). In the present study the effect of AMF on biomass production, osmoregulation and antioxidant metabolism was investigated in date palm seedlings under water deficit.

MATERIALS AND METHODS

Pre-germinated seeds of date palm were transferred in pots containing 1 kg of sterilized soil collected from date palm grove and grown under greenhouse conditions. Half of plantlets were inoculated (AM-plant) with 10g of rhizospheric soil containing hyphae, mycorrhizal root fragments, and spores of the AM fungus *Glomus intraradices* recognized from earlier investigations as efficient for promoting date palm growth and nutrition (Baslam *et al.,* 2009). The same amount of autoclaved inoculum was added to non-inoculated plants (N-AM). Water stress treatments consisted of two watering regimes: 75% of field capacity (well water) and 25% of field capacity (water stress). Water status of the pots was daily examined and the amount of water loosed was refilled into each pot. The experiment was arranged in a completely randomized block design. Each treatment was replicated twenty times. Eight weeks after water stress application, plants were harvested and roots were washed free from soil under a stream of cold tap water. Root colonization (%M) was evaluated according to Trouvelot et al. (1986). Shoot height (SH) and root length (RL) were measured and shoot (SDM) and root (SDR) dry matters were recorded by drying in oven at 70ºC to constant weight. Biochemical changes including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) activities and osmoregulation matters including proline, soluble protein and total soluble sugar (TSS) were determined according to balsam *et al.,* (2009). Leaf antioxidant enzyme activities, SOD (Beyer et Fridovich, 1987), CAT (Aebi, 1984), APX (Nakano and Asada, 1981) and GPX (Maehly and chance, 1954) were determined. Malondialdehyde (MDA) was measured by the thiobarbituric acid method as described by Heat and Packer (1981) and H_2O_2 by using titanium method according to Patterson and al. (1984). TSS was analyzed with the anthrone method (Irigoyen *et al.,* 1992) and proline with the ninhydrin reaction according to Bates et al. (1973). 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

Date palm plants inoculated with AMF (AM-plants) showed mycorrhizal structures in roots while these al structures are never seen in roots of non-inoculated plants (N-AM). Water restriction disfavored the colonization of date palm roots by AMF (Table 1). Percentages of mycorrhizal colonization reached 61 % in well watered (WW) plants comparing to 42% in water stressed (WS) ones. The mycorrhizal efficiency index (ME)I increased in AM-plants cultivated under reduced irrigation regime compared with their respective WW N-AM plants (table 1). AMF inoculation notably increased date palm shoot height (SH) and root length (RL) and shoot (SDM) and root dry matter (RDM) regardless water regime (Table 1). Water stress significantly decreased plants growth (SH and LR) and biomass production (SDM and RDM), this decrease was more important in N-AM plants.

Water stress increased the MDA concentration of leave of both N-AM and AM-plants (Table 2). The increase of MDA content in response to WS was more relevant in N-AM (23%) than in AM-plant (9%). There was higher total soluble sugar and proteins and high proline concentration in leaves of AM-plant under WS than in their respective N-AM plants. Analyses of hydrogen peroxide revealed that the level of H2O2 was increased by drought in N-AM plants. In contrast, concentrations of H2O2 were similar in leaves of AM-plants under both WW and WS conditions (Table 2). Whether WS or not, AM symbiosis notably increased GPX and APX activities of leaves (Table 2). AM colonisation also markedly increased SOD activity and slightly decreased CAT activity of WS leaves (Table 2).

DISCUSSION

AM symbiosis increased growth and biomass production of date palm plants under both well water presence and water stress condition, confirming earlier findings (Meddich, 2000; Baslam and al., 2008, 2009; Aqqua *et al.,* 2010; Faghire *et al.,* 2010; Baslam *et al.,* 2013). The positive effect of AMF is likely attributed to the improvement of mineral nutrition, the enhancement of water uptake and the increase of root length density.

Osmotic adjustment due to the accumulation of certain organic and inorganic molecules osmotically active in plant cells is one of the mechanisms of the tolerance of water stress. Our results showed that soluble sugar and proline

levels in leaves were higher in water stressed AM-plants than those in corresponding N-AM. Such elevated level of proline and TSS accumulation in water stressed AM-plants was reported by many previous investigations (Fouad *et al.,* 2012, 2013; Baslam *et al.,* 2013). These authors showed that proline and sugars accumulation played a role in osmotic adjustment and allowed cells to maintain turgid and the processes that depend on it, such as cellular expansion and growth.

In higher plants, ROS production and removal are strictly controlled under amply watered conditions (Apel and Hirt, 2004). When higher plants are subjected to water stress, the equilibrium between production and scavenging of ROS is broken, resulting in oxidative damage to proteins, DNA and lipids. The oxidation of membrane lipids is a reliable indication of uncontrolled free-radical production and hence of oxidative stress (Noctor and Foyer, 1998). Many reports have emphasized the importance of AMF in increasing antioxidant activity and reducing oxidative damage (Ruiz-Lozano 2003; Alguacil et al. 2003). Accordingly, H_2O_2 accumulation and oxidative damage estimated as the ratio of malondyaldehide to proteins in mycorrhizal date palm seedlings subjected to drought was three times lower than in their respective non inoculated seedlings. Additional biochemical responses including enzymatic defense is an important component of the protective systems that minimize the deleterious effect of water stress. SOD catalyses the dismutation of O2 to H2O2, CAT dismutates H2O2 to oxygen and water, and APX reduces H2O2 to water. Our result showed that AM symbiosis notably increased the activity of GPX, APX and SOD and decreased the activity of CAT in date palm seedlings under water. Our finding suggest that the increased activity of antioxidant enzymes and decreased concentration of ROS compounds found in AM plants may serve to protect the date palm against oxidative damage, enhancing drought tolerance. These results are in good agreement with previous investigations showing that AMF inoculation markedly enhances the antioxidant enzyme activities (GPX, SOD and APX) and steeply reduces MDA and H_2O_2 accumulation (Alguacil *et al.,* 2003). Arafat and He (2011) associated the lower accumulation of H_2O_2 and lipid peroxidation, evaluated by MDA production, with the greater activity of antioxidant enzymes in AMF compared to non AMF plants. Other authors have shown a positive correlation between tolerance to water deficit and increased antioxidant activities (Ruiz-Lozano, 2003; Alguacil and al., 2003).

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Tables

Table 1: Root colonization (%), Plant height (cm), Root length (cm), shoot and root dry matters (g) of non-mycorrhizal (N-AM) or mycorrhizal (AM-plant) date palm seedlings grown under well water (75%FC) or water stress (25%FC) conditions.

Water regime	AM status	M	SDM	RDM	SH	RL	MEI
75%FC	N-AM	0	3.16c	1.76d	22.50c	27.90d	ND
	AM-plant	61.43a	6.41a	3.38a	44.67a	60.50a	49.7b
25%FC	N-AM	0	1.43e	0.81e	13.33d	18.95d	ND
	AM-plant	42.6c	3.21c	2.97b	26.60c	46.90c	63.8a

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2: Total soluble sugar (mg.g^{-1} DM), hydrogen peroxide (mmol.g^{-1} DM), proline (nmol.g^{-1} DM), malonyldialdehyde (nmol.g^{-1} DM) and protein (mg.g^{-1} DM) contents in leaves of non-mycorrhizal (N-AM) or mycorrhizal (AM-plants) date palm plants grown under well watered (75%FC) or water stress (25%FC) conditions.

Water regime	AMF status	TSS	H_2O_2	Prolin	MDA	Protein
75%FC	N-AM	54.76c	25.54b	4105.2c	53.23b	6.14a
	AM-plant	78.76a	26.2b	9193.2a	38.61d	5.75a
25%FC	N-AM	47.53d	28.58a	3570.2d	65.51a	3.3c
	AM-Plant	68.51b	26.53b	4070.4c	42.10c	4.21b

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).

Table 3: Catalase (nmol mg^{-1} prot), superoxide dismutase (U mg^{-1} prot), ascorbate peroxidase (mmol.mg^{-1} prot), and guaiacol peroxidase (mmol.mg^{-1} prot) activities in leaves of non-mycorrhizal (N-AM) and mycorrhizal (AM-plants) date palm plants grown under well water (75%FC) or water stress (25%FC) conditions.

Water regime	AMF status	SOD	GPX	CAT	APX
75%FC	N-AM	452.3c	3.07c	62.7cd	2.11d
	AM-plant	447.7c	4.20b	136.8c	3.89c
25%FC	N-AM	596.1b	4.44b	225.5a	4.68b
	AM-plant	667.4a	5.87a	114.41d	7.05a

Within each column, values followed by the same letter are not significantly different ($p \leq 0.05$).

Status of arbuscular mycorrhizal fungi in different plants of date palm plantation of Al-jamil farm at Qassim, Saudi Arabia

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ABSTRACT

Arbuscular mycorrhizal fungal association were assessed in different plant species namely Allium sativum, Cenchrus ciliaris, Cynodon dactylon, Helianthemun sp, Malva parviflora, Medicago sativa, Trigonella foenumgraceum, and Zea mays collected from date palm plantation of Al-jamil Farm at Qassim, Saudi Arabia. Roots and rhizosphere soils were processed by following the standard methods. Total colonization, spore population and diversity significantly varied in different plant species. Total mycelial colonization varied from 28-72% with the highest in A. sativum (72%) and the lowest in Helianthemum sp. (28%). Maximum vesicular colonization was in Zea mays (60%) and minimum was in Cynodon dactylon(31%). The highest arbuscular colonization was found in Trigonella foenumgraceum (59%) and the lowest was in Helianthemun sp. (26%). Arbuscular mycorrhizal fungal spore population varied 95-130/100g dry soil. The highest number was recorded in Trigonella foenumgraceum (130) and the lowest was in Medicago sativa (95). Funneliformis mosseae (8-42%), Glomus etunicatum (5-26%), Glomus intraradices (14-50%), Glomus sp (3-21%). Gigaspora sp. (8-62%) and Sclerocystis sp. (4-42%) were identified from the rhizosphere soils. A few spores were unidentified.

Keywords: Arbuscule, Colonization, Diversity, Mycorrhiza, Mycelium, Vesicles, Spore population.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the firstborn perennial fruit trees and it stands tall with their branches outstretched and their roots anchored deep into the earth in the sanctuaries of Saudi Arabia. Date palms have been a treasured part of the Saudi landscape for their beauty as well as their utility. Since ancient times, the date palm has been a source of food for Arabian people, and its branches provide with shade from the sturdy desert sunlight. The Kingdom of Saudi Arabia is the world's second largest producer of dates, supplying 17.6 percent of the world market. The estimated annual production of dates in Saudi Arabia is 1,008,105 tons occupying an area of 156,023 hectares with 23,742,593 date palm trees (MOA, 2012).

In the arid ecosystems of Saudi Arabia, drought is an important abiotic factor and liable for limiting plant growth and yield (Kramer and Boyer, 1997). Plants growing in these stressed conditions form mutualistic mycorrhizal interaction to survive the drought stress (Auge, 2001; Ruiz-Lozano *et al.,* 2001) and to adapt themselves by their morphological, anatomical and physiological responses (Bray, 1997) in addition. Arbuscular Mycorrhiza Fungi (AMF), belonging to Phylum-Glomeromycota (Redecker *et al.,* 2013), are important constituents of the soil microbial community in terrestrial ecosystems forming mutualistic symbiotic association with most of the terrestrial plants (Trappe, 1987). AMF have been shown to promote plant growth by uptake of slow releasing nutrients (Newsham *et al.,* 1995), drought

tolerance (Auge, 2001), salinity tolerance (Evelin *et al.,* 2009), establishment and growth in harsh environments (Koske and Polson, 1984), protection to roots against soil borne pathogens (Azcon-Aguilar and Barea, 1996), improve host physiological processes, promote plant diversity (van der Heijden *et al.,* 1998) etc. AM fungi are important to the persistence of vegetation in harsh environment conditions. However, little is known about the biodiversity of AM colonization and spore population in Saudi vegetation (Khaleil, 1989; Malibari *et al.,* 1990; Al-Garni, 2001; Al-Whaibi, 2009; see also Al-Qarawi *et al.,* 2012). The mycorrhizal association of different crops and weeds in various agro-ecosystems are well known (see Muthukumar and Prakash, 2009), no specific reports on mycorrhizal association in different plants growing in the farming systems of Saudi agro-ecosystems. The present study was undertaken to test our hypothesis that plant species growing in various farms of Saudi agro-ecosystems may demonstrate the arbuscular mycorrhizal association. We examined different plants growing in the date palm plantation at Qassim, Saudi Arabia for their arbuscular mycorrhizal association and diversity of arbuscular mycorrhizal fungi in the soil.

MATERIALS AND METHODS

Root and soil sample collection and preservation

Different agricultural and non-target plants were growing in the date palm plantations of Qassim. Roots and rhizosphere soil samples of *Allium sativum, Cenchrus ciliaris*, *Cynodon dactylon*, *Helianthemun* sp, *Malva parviflora*, *Medicago sativa*, *Trigonella foenumgraceum* growing in the date palm plantation were collected. Removing the gravels from top soils, roots and rhizosphere soils were collected from 5−30 cm soil layer. Roots were preserved in 50% alcohol after cleaning and washing. Preserved roots were cleaned, chopped into 1cm pieces and stained with 0.05% aniline blue (Philips and Hayman, 1970; Koske and Gemma, 1989) with modifications. Assessment of AM colonization was followed under digital computerized microscope. Data were recorded on total colonization, intensity (poor, moderate and abundant) (Dhar and Mridha, 2006) of AM structural (mycelium, vesicle and arbuscule) colonization. Mycelial colonization was regarded as total colonization. Percent colonization and intensity of AM structural colonization were calculated (Dhar and Mridha, 2006).

Processing of soil samples and spore isolation

From each sample, 100g soil was processed by wet sieving and decanting method (Gerdemann and Nicolson, 1963) with some modifications. The series of ASTM-60, ASTM-100, ASTM-270 and ASTM-400 sieves were used to extract the spores. Part of residues on the sieves was used for isolation of the spores through centrifugation with 60% sugar solution and other part was used for filtration method to have intact spores with morphological structures (Gerdemann and Nicolson, 1963). Spore suspension was filtered through gridded Whatman filter paper No-1 facilitating the easy counting of the spores. After filtration the paper was examined under the stereo-binocular microscope at 2.5´10 magnification and spore number was recorded. The total number of spore population in each individual sample was calculated per 100g dry soil basis.

Identification of AMF spores

Morphologically similar spores were separated and observed under computerized compound microscope mounting on PVLG and Melzer's reagent to identify by following the established literatures (INVAM, 2013; Schenck and Perez, 1990; Schüßler and Walker, 2010; Redecker *et al.,* 2013). Total spore population, species richness and Shannon's diversity index (Hs) of AM fungal species were calculated (see Dhar and Mridha, 2006).

Statistical analysis

Data were analyzed by One Way Anova and means were compared using the SPSS 21 at 0.05% level.

RESULTS

Data on the arbuscular mycorrhizal (AM) colonization have been presented in the Table-1. The range of AM colonization varied from 28-72%. The highest was recorded with *Allium sativum* (72%) followed by *Zea mays* (70%) and *Trigonella foenumgraceum* (69%). Vesicular colonization was recorded 31-60%. Maximum vesicular colonization was recorded in *Zea mays* (60%) which was followed by *Trigonella foenumgraceum* (58%). Minimum was recorded in *Cynodon dactylon* (31%). Arbuscular colonization was observed 26-59%. The highest arbuscular colonization was found in *Trigonella foenumgraceum* (59%) and the lowest was in *Helianthemun* sp (26%). Intensity of mycelial, vesicular and arbuscular colonization varied independently (Table-1).

Data on the total AM fungal spore population and AM fungal species richness in the rhizosphere soil of different plant species under study have been presented in the Table-2. The highest AM fungal spore population was counted in the rhizosphere soil of *Trigonella foenumgraceum* (130) which was followed by *Zea mays* (125)*, Allium sativum* (121) and *Helianthemun* sp (116). The lowest spore population was counted in the rhizosphere soil of *Medicago sativa* (95). *Funneliformis mosseae, Glomus etunicatum, Glomus intraradices, Glomus fasciculatum, Glomus* sp*, Gigaspora* sp*, Sclerocystis* sp were identified. *Funneliformis mosseae* was recorded 8-42% in six samples: maximum in *Cenchrus ciliaris* (42%) and minimum in *Medicago sativa* (8%).

Glomus etunicatum was observed 5-26%. The highest was recorded in the sample of *Malva parviflora* (26%) and the lowest was in the sample of *Allium sativum* (5%). Glomus sp-1 was counted 3-21%. *Gigaspora* sp was observed in five soil samples (5-62%). The highest population was recorded in the soil sample of *Medicago sativa* (62%) and the lowest was recorded in the soil sample of *Cynodon dactylon* (5%). *Sclerocystis* sp was recorded 4-42% from five soil samples. Maximum was in the soil of *Trigonella foenumgraceum* (42%) and minimum was in the soil of *Malva parviflora* (4%). A few spore remained unidentified.

Figure-1 represents the data on the Shannon's diversity index (Hs) of AM fungi in the rhizosphere soils of different plants growing in the date palm plantation of Al-Jamil Farm at Qassim of Saudi Arabia. The highest Hs was calculated in the soil of *Helianthemun* sp and it was followed by *Cenchrus ciliaris, Cynodon dactylon* and *Malva parviflora.* The lowest was in the soil sample of *Medicago sativa*.

DISCUSSION

All the plant species under study were observed to be associated with arbuscular mycorrhizal fungi. Occurrence of different arbuscular mycorrhizal fungal species has been confirmed in the date palm plantation of Al-Jamil Farm at Qassim, Saudi Arabia. Different AM fungal structures viz: coenocytic mycelium, vesicles and intracellular arbuscules were observed and their presence confirmed the occurrence and association of arbuscular mycorrhizal fungi with the growing plant species in the date palm plantation. *Allium sativum, Trigonella foenumgraceum* and *Zea mays* were observed to be highly colonized. They have no significant difference in case of total colonization. These agricultural crop plants were previously reported to be highly mycorrhizal (Christopher and Vyan, 2008; Chu et al, 2013; Gill *et al.,* 2013; Tuncturk, 2011). Variation in total AM colonization was significant among other plants species. Similar variation of AM colonization in different desert plants were reported in the earlier studies (see Al-Qarawi *et al.,* 2012). Arbuscular colonization in the plant species indicates the active role of mycorrhizal symbionts in the date palm plantation. Intensity of mycelial, vesicular and arbuscular colonization were variable in different plant species.

Total spore population was variable in all soil samples which was statistically significant ($p<0.05$). Most of the AMF species were under *Glomus*. Arbuscular mycorrhizal fungal species richness and distribution varied independently in different rhizosphere soil samples. It may remarkably be mentioned that agricultural crops growing in the date palm plantation showed lower species richness and diversity index. It may be due to the different cultural practices in the agro-ecosystem. It is reported that cultural practices may be responsible for lower species richness, spore density and diversity of AM fungi (Abbott and Robson, 1991). Whereas naturally growing non-target plants showed comparatively higher species richness and diversity index. Such phenomenon may be the result of the plant's survival strategy in the adverse condition.

Funneliformis mosseae was also recorded. Different recorded species of *Glomus* were more or less similar in all the samples. Frequency distribution (species richness) of different AMF species varied significantly. It is notable that *Gigaspora* spp were higher *Glomus* spp were almost lower in the agricultural plants and whereas in the non-target plants *Gigaspora* sp was lower and *Glomus* was frequently occurring. The reason of this inverse interaction is unknown. More and extensive studied are emphasized hereby to understand any positive or negative interrelationship between *Gigaspora* and *Glomus* in the agroecosystem at Qassim of Saudi Arabia. Species composition of AMF community may be influenced by the host species (see Kumar *et al.,* 2012). Growth and development habits of the host and AM fungi may also influence the variation of AMF structural colonization and sporulation of the fungal symbionts (see Kumar et al,.2012). Diversity of AM fungal species is important in the soil systems for the best nutrient uptaking.

Arbuscular mycorrhizal fungi spread extraradical mycelia network in the pedosphere binding the microparticles by filamentous hyphae constituting persistent aggregates of the soil particles. Stability of soil aggregates is related to the soil density, root length and extra-radical mycorrhizal mycelium in the rhizosphere soils (see Graf and Frei, 2013). Mycorrhizal fungal mycelial mass in the soils of date palm farm increase soil stabilization and thus reduce the soil erosion in Saudi agro-ecosystems. Arbuscular mycorrhizal inocula are dispersed by the windflow in the desert fields and they are deposited near the gravels on the soil surface. New borne seedling of different non-target plants facilitate the colonization and spreading of these deposited mycorrhizal propagules (Koske and Polson, 1984) which help to nutrient enrichment, soil improvement, and microbial development in the date palm rhizosphere thus favouring the date palm trees.

In the abiotically stressed habitats like arid ecosystems of Saudi Arabia, AM dependent plants acquire mycorrhizal symbiosis for their survival and nutritional requirement. Estimation of AM fungal inoculum potential in the non-target plants and their influence in the nutrient cycling, nutrient management, and maintenance of the balanced soil health of the date palm plantation in the harsh conditions of Al-Qassim might be important. As such these non-target plants, supporting the date palm trees by maintaining the diversity of AMF species, providing with sufficient nutrients and managing the soil health system, may be regarded as helpful friends to the date palm trees in the abiotically

stressed agroecosystems of Saudi Arabia with the importance of nutritional, ecological and evoltuionary standpoint.

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Tables

Table 1: Total colonization and intensity of different AM structural colonization in the roots of various plants species growing in the date palm plantation of Al-Jamil farm at Qassim, Saudi Arabia.

Plant species	Total Colonization (%)			Intensity of AM structural colonization (%)								
				Mycelium			Vesicles			Arbuscules		
	Mycelium	Vesicles	Arbuscules	P**	M	A	P	M	A	P	M	A
A. sativum	72 a*	42 c	27 e	42	41	17	53	43	4	32	34	34
C. ciliaris	45 d	40 c	36 c	27	53	20	38	47	15	35	52	13
C. dactylon	52 c	31 d	31 d	25	75	--	--	100	--	100	--	--
Helianthemun sp	28 e	35 d	26 e	32	47	21	46	54	--	47	51	2
M. parviflora	66 b	53 b	48 b	25	46	29	32	62	6	50	24	26
M. sativa	46 d	42 c	33d	24	53	23	54	23	23	25	53	22
T. foenumgraceum	69 ab	58 a	59 a	21	57	22	54	40	6	15	53	32
Z. mays	70 ab	60 a	57 a	36	43	21	20	46	34	--	100	--

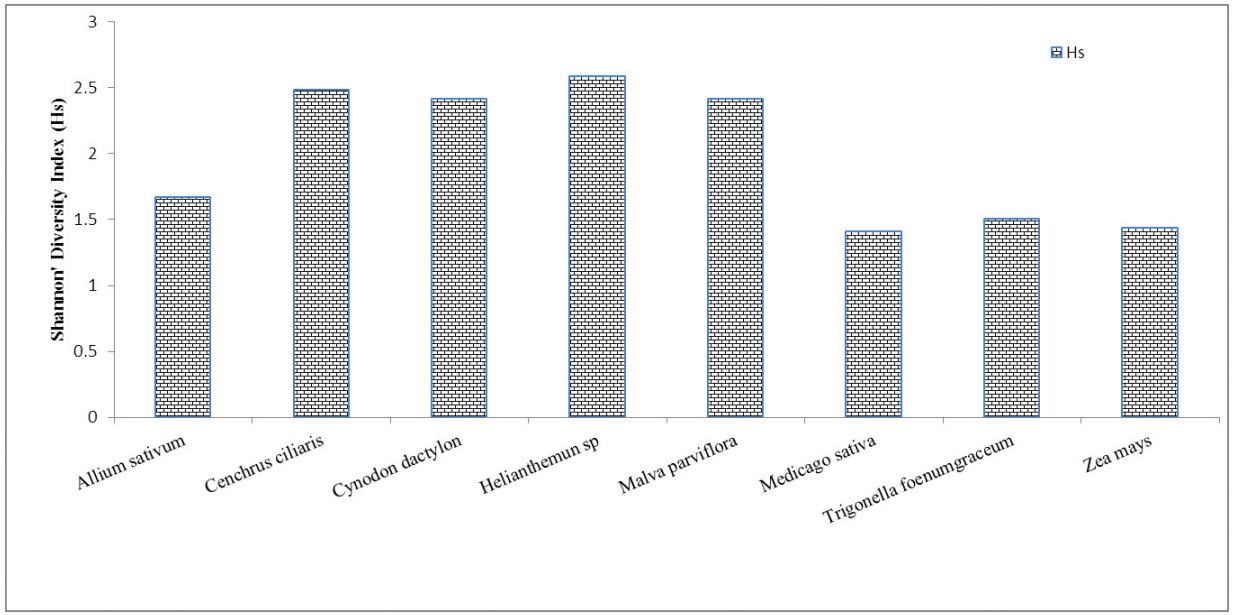
*Different letters indicate the significant variation as shown by DMRT ($p<0.05$).
**P-Poor, M-Moderate, A-Abundant.

Table-2: Total spore population and percent population of different AM fungal species in the rhizosphere soil of various plant species growing in the date palm plantation of Al-Jamil farm at Qassim, Saudi Arabia.

Plant species	Total population	% population of different AM fungal species						
		F. mos**	G. etu	G. intra	Glom. sp.-1	*Gig.*	*Scl.*	Unidfd
A. sativum	121 c*	35	5	50	3	--	--	7
C. ciliaris	105 e	42	14	20	11	8	--	5
C. dactylon	97 g	38	21	14	13	5	--	9
Helianthemun sp	116 d	21	21	22	12	--	5	19
M. parviflora	102 f	25	26	19	21	--	4	5
M. sativa	95 g	8	--	--	--	62	26	4
T. foenumgraceum	130 a	--	--	--	19	39	42	--
Z. mays	125 b	--	--	--	16	52	32	--

*Different letters indicate the significant variation as shown by DMRT ($p<0.05$).
**F. mos =*Funneliformis mosseae*, G. etu =*Glomus etunicatum*, G. intra =*Glomus intraradices*, Gig.=*Gigaspora* sp, Scl.=*Sclerocystis* sp., Unidfd=Unidentified.

Figure:



Shannon's Diversity Index (Hs) of AM fungi in the soil samples of different plants growing in the Date Palm Plantation

Accelerated ripening of var. Aseel dates fruit using sodium chloride and acetic acid solutions

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ABSTRACT

Pakistan is rated among the top dates producing countries. The ripening and harvesting time of var. Aseel which is the predominant variety of district Khairpur is often coincides with Monsoon rains that deteriorating most of the crop either the fruit while on tree or on mats for sun drying. Due to this fear growers harvested the fruit at khalal stage and make Chuhharas (boiled date fruit at khalal stage) that has less commercial value than tamar fruits exporting worldwide. Instead of making Chuhharas, artificial ripening of var. Aseel fruit was accelerated by using sodium chloride (NaCl) and acetic acid (CH_3COOH) solutions to treat the early harvested and unripe fruit to reach tamer stage by skipping rutab stage. In current study, the harvested fruit at Khalal stage were dipped in different concentrations of both solutions for 4-5 minutes at room temperature. The collected fruit were spread out in a single layer on stainless steel trays for sun curing. Physical and chemical characters were measured before dipping treatments in both solutions and after 72 and 120 hours of sun curing. The obtained results showed the cured fruit dipped in 2% sodium chloride (T_4) was found to possess high amount of pulp and total soluble solids. It also hold large size & weight and furnish high yield of very appealing organoleptic qualities.

Keywords: Accelerated ripening, sodium chloride, Aseel dates, acetic acid.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an important horticultural crop and cultivated in the warmer regions of the world mainly concentrated in Muslim countries like Egypt, Iran, Saudi Arabia, Pakistan, UAE, Oman and Libya. Date fruits being sweet and most nutritious supplying 2500-3000 Calories/kg (Amin, 2007) consumed as a staple food because the sugar content of ripe dates is about 80%; the remainder consists of protein, fat and mineral products including copper, sulphur, iron, magnesium and fluoric acid. Dates are high in fiber and an excellent source of potassium.

Date occupies third position after citrus and mango in terms of fruit area and production in Pakistan (Khushk *et al.,* 2009). The export of dates is mainly occurring from Khairpur and Turbat districts of Pakistan. There is a big gap between production and export figures. Pakistan on an average export 10-15 % of dates production and 85-90 % crop production is either consumed locally or wasted (Jatoi *et al.,* 2009).

The Aseel variety is a semi-dry and most important commercial variety of Pakistan mainly found in Khairpur district (Fig. 1). It with suitable fruit size (4.3 cm in length and 2.5 cm in diameter) consumed at rutab (Dang) and tamar (dates) stages (Markhand *et al.,* 2010; Mahar, 2007).

Each year, monsoon clashes with the dates ripening season at Khairpur and a few hours of rain may wipes out crop because rain water percolates inside the fruit bunch if fruit still on tree ready for harvest or on mats for drying (Fig. 2)

gets fermented and total losses may rise to as high as 50% (Saleem, 2004; Saleem *et al.,* 2005). To avoid unexpected risks during monsoon rains, growers are compelled to process un-ripened and non-edible var. Aseel fruit into low-priced 'chuhharas' before the start of monsoon season. The use of centuries-old unhygienic practices and hesitation in using modern techniques deteriorates the product quality, slow the growth of export and badly affects the growers' income. Such practices further contribute towards 20% to 30% post-harvest losses each year.

In order to avert such heavy losses resulting from the natural calamities and produce high quality product, present study was conducting to explore ways and means to ripe/cure var. Aseel dates artificially by using sodium chloride and acetic acid solution. Farahnaky et al. (2009) found that harvesting var. Kabkab dates at the Khalal stage followed by a short-time dipping of the fruits in NaCl or acetic acid solutions and an incubation stage of about 48-72 hours at 40°C is a promising method for controlled ripening of date fruits. This specific procedure accelerates ripening of the date fruits from khalal to tamar stages to over three days instead of weeks of natural ripening process on the tree.

MATERIALS AND METHODS

The research work has been conducted at Date Palm Research Institute, SALU, Khairpur. Healthy and uniform var. Aseel fruits were collected from commercial date palm orchard in Khairpur (Northern Sindh) at the khalal stage in July 2012. The selected dates were wiped with moist clean cloth to remove the dust. Then, they were picked out and randomly distributed into 13 batches (one kilogram each) and given preliminary treatments of NaCl and acetic acid (Fig. 3). The codes and composition of the treatments are given in Table 1.

The samples were immersed in the respective solutions (one liter) for 4-5 minutes at room temperature, allowed to drain and then spread out separately on stainless steel trays for dehydration under sun shine.

Data Collection

Physical and chemical characteristics were monitored before experiment at khalal stage (Table 2) and after 72 and 120 hours (Table 3, 4 & 5). Moisture content, pH and total soluble solids (brix) were quantitatively determined according to AOAC methods. Color and texture were observed and recorded after 72 and 120 hours.

Statistical analysis

The treatments were performed using a completely randomized design and all experiments were carried out in five replicates. The experimental data were subjected to analysis of variance followed by a multiple range Duncan's test. Significance was defined at P = 0.05. The SPSS (developer, 13) program was used for all statistical analysis.

RESULTS AND DISCUSSIONS

The efficacy of sodium chloride and acetic acid for initiation/acceleration of ripening of var. Aseel dates has been investigated. Each treatment was applied individually and in combined form at different proportions varying from 0.25 to 3.5% and 0.25 to 2.5% for sodium chloride and acetic acid respectively. Var. Aseel dates harvested at the khalal stage were immersed in solutions for 4-5 minutes and allowed to ripen/cure for 72 and 120 hours in sun shine for dehydration. Observing changes in color shade, fruit weight, pulp, texture, total soluble solids, appearance and the extent of the ripening assessed the efficiency of the treatment. All of the treatments whether applied as a single treatment or in combined form, tend to induce ripening by causing changes in the selected quality parameters.

Physical characteristics

The results indicated that harvesting var. Aseel fruits at the khalal stage followed by a short-time dipping in NaCl or acetic acid solutions at incubation period 72 and 120 hours is a promising method for controlled accelerated ripening of date fruits. This specific procedure accelerated ripening of the date fruits from khalal to tamar stages within 3 - 5 days instead of weeks by traditional sun curing method.

The data presented in table 3 in comparison with table 2 shows that the fruit and pulp weight dramatically decreased from khalal to tamar stage at 72 and 120 hours incubation period. The highest fruit weight recorded on T_{13} (6.98 g) followed by T_4 (6.86 g) and T_2 (6.46 g) at 72 hours while lowest was observed at 120 hours incubation period on T_{11}. The highest pulp weight was noted on T_{13} (6.22 g) followed by T_4 (5.86 g) and T_7 (5.76 g) at 72 hours, while lowest on T_1 (3.14 g) at 120 hours. The highest seed weight was recorded on T_4 (1.24 g) followed by T_5 & T_6 (1.1 g) and T_1 (1.06 g) at 72 hours, while lowest on T_{10} (0.64 g) at 120 hours. The maximum length was recorded on T_4 (3.82 cm) followed by T_3 & T_6 (3.22 cm) and T_9 (3.2 cm) while lowest on T_1 (2.70 cm) at 120 hours. The maximum fruit width was observed on T_4 (1.84 cm) followed by T_8 (1.64 cm) and T_7 (1.62 cm) at 72 hours, while lowest on T_{11} (1.30 cm) at 120 hours incubation period.

Generally, salt treated fruits responded well in all physical parameters of var. Aseel when used alone or in combination with acetic acid than control and acetic acid alone. Actually, fruits treated with NaCl does not undergo at dung (rutab) stage hence saving the 2 weeks' time period as compared to traditional sun drying method. These results are in agreement with the findings of Saleem

(2005) who used NaCl and acetic acid solution for the accelerated ripening of Dhakki date fruits and found NaCl better than other control and acetic acid treatments.

Chemical Characteristics

The data presented in table 4 clearly indicating that the moisture content of the date samples reduced during incubation from khalal to tamar at 72 and 120 hours' time period. The highest moisture content was recorded on T_{13} (85.00%) followed by $T_{10} - T_{12}$ (81.00%) at 72 hours, while lowest on T_2 (15.00%) at 120 hours incubation period. Ali (1989) studied the effect of hot solutions on the curing of dates and concluded that the fruit undergoing storing lost its weight through moisture evaporation.

One of the important parameters determining the microbial stability and hence the shelf life of date fruits is their pH. The highest pH value was recorded on T_5 (7.10) followed by T_5 (7.03) and T_9 (6.92) while lowest on T_4 (4.10) at 120 hours incubation period.

The highest amount of TSS was recorded on T_8 & T_{10} (4.90) followed by T_5 & T_{11} (4.10) at 120 hours, while lowest on T_1, T_3, T_8 and T_9 (2.10) at 72 hours incubation period.

Color and Texture characteristics

The results presented in table 5 showing the color and texture of fruit at 72 and 120 hours of curing. The color of fruit plays a pivotal role in the marketing value and quality index. Similarly variation in the color is closely associated with ripening of fruit (Farahnaky *et al.,* 2009). All the treatments exerted a positive effect on the color and texture of fruit. However both the characters varied with the nature of treatment applied (table 5). The amber color with attraction and shinning was recorded in T_4 with soft and very loose texture of fruit. This color and texture was considered the best in the produced lot of colors and texture. Amber color was also developed by some other treatments like T_1, T_{10} and T_{11} but it wasn't attractive and shinning. These findings are in agreement with the results on Dhakki dates by Saleem et al. (2005).

In current study time for incubation after dipping treatment in the tested solutions was increased up to 120 hours as the results obtained by Farahnaky et al. (2009) reported that harvested dates fruit of var. Kabkab at the Khalal stage followed by a short-time dipping of the fruits in NaCl or acetic acid solutions and an incubation stage of about 48-72 hours at 40°C is a promising method for controlled ripening of date fruits. The same findings have been produced by Saleem et al. (2005) while working on Dhakki dates in Pakistan. On the contrary, current study results indicated that 120 hours treatment proved better than the 72 hours treatment for var. Aseel. The difference in curing time from 72hours for Kabkab and Dhakki and 120 hours for var. Aseel could be because of varietal difference.

CONCLUSIONS

It can be concluded from the results obtained that sodium chloride and acetic acid exerted a positive response on ripening of var. Aseel dates by accelerated curing process. Using, 2% sodium chloride proved more effective in terms of fruit weight, pulp contents, color, texture, taste and appearance (Fig. 4). It proves beyond doubt that leaving the fruit on the tree to get dung (Rutab) formation through natural process is not justified that taken at least six weeks for the conversion of fruit from khalal to tamer stages. Whereas, the same process of conversion has been accelerated by artificial means through bypassing the Rutab stage within 120 hours using NaCl and acetic acid solutions. Hence, suggested a possible solution against making low price chuhhara by skipping the fear of monsoon rains by accelerating the ripening process of var. Aseel dates.

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Tables

Table 1. The abbreviations and composition of different treatments used in the study.

Treatment	Solution composition
T_1	Water (control)
T_2	0.25%NaCl
T_3	1.50%NaCl
T_4	2.00% NaCl
T_5	3.50%NaCl
T_6	0.25%CH3COOH
T_7	0.50% CH3COOH
T_8	1.50% CH3COOH
T_9	2.50% CH3COOH
T_{10}	2.00%NaCl+0.25%CH3COOH
T_{11}	2.00%NaCl+0.50%CH3COOH
T_{12}	0.25NaCl+1.50% CH3COOH
T_{13}	2.00%NaCl+2.50%CH3COOH

Table 2. Physical and chemical characteristics of var. Aseel fruits at khalal stage before treatments.

Color	Texture	Length (cm)	Width (cm)	Fruit wt (gm)	Pulp wt (gm)	Seed wt (gm)	T.S.S	pH	Moisture (%)
Yellow	firm	3.56	2.34	11.76	9.86	1.90	2.00	6.9	86

Table 3. Effect of sodium chloride and acetic acid treatments for 72 and 120 hours on the physical characteristics of var. Aseel dates fruit.

Treatments	72 (hours)					120 (hours)				
	Fruit weight (g)	Fruit Length (cm)	Fruit Width (cm)	Pulp weight (g)	Seed weight (g)	Fruit weight (g)	Fruit Length (cm)	Fruit Width (cm)	Pulp weight (g)	Seed weight (g)
T_1 (control)	6.12	2.84	1.48	4.88	1.06	4.18	2.70	1.46	3.14	0.78
T_2	6.46	3.08	1.58	5.50	0.96	4.84	2.98	1.44	3.66	0.90
T_3	6.16	3.22	1.54	5.30	1.02	4.18	2.76	1.39	3.54	0.80
T_4	6.86	3.82	1.84	5.86	1.24	5.24	3.06	1.50	4.36	0.88
T_5	5.46	3.08	1.58	4.52	1.1	5.22	3.06	1.44	4.28	0.92
T_6	5.36	3.22	1.38	4.58	1.1	4.96	3	1.42	3.68	0.80
T_7	6.56	2.84	1.62	5.76	0.80	4.56	2.92	1.44	3.60	0.84

Treatments	72 (hours)					120 (hours)				
	Fruit weight (g)	Fruit Length (cm)	Fruit Width (cm)	Pulp weight (g)	Seed weight (g)	Fruit weight (g)	Fruit Length (cm)	Fruit Width (cm)	Pulp weight (g)	Seed weight (g)
T_8	6.42	3.00	1.64	5.6	0.80	4.76	2.94	1.50	3.88	0.96
T_9	6.42	3.2	1.54	5.60	0.82	4.72	2.88	1.34	3.92	0.80
T_{10}	6.56	3.2	1.50	5.74	0.76	4.32	2.76	1.34	3.56	0.64
T_{11}	5.08	3.16	1.50	4.38	0.70	3.62	2.84	1.30	3.40	0.88
T_{12}	6.12	3.16	1.6	4.98	0.78	4.94	3	1.56	4.12	0.74
T_{13}	6.98	3.04	1.62	6.22	0.76	4.84	2.88	1.52	4.08	1.3
LSD at 0.05	**1.26**	**0.36**	**0.36**	**1.18**	**0.43**	**1.77**	**0.40**	**0.75**	**1.77**	**0.16**

Table 4.Effect of sodium chloride and acetic acid treatments for 72 and 120 hours on the chemical characteristics of the var. Aseel dates fruit.

Treatments	72 (hours)			120 (hours)		
	Moisture%	pH	T.S.S	Moisture%	pH	T.S.S
T_1 (control)	72.60	6.80	2.10	16.00	6.55	3.80
T_2	74.30	6.30	3.20	15.00	6.09	3.70
T_3	66.00	6.40	2.10	17.00	6.84	3.80
T_4	66.00	6.60	3.20	16.00	4.10	4.00
T_5	60.00	6.51	2.50	16.00	7.10	4.10
T_6	65.00	6.80	2.20	16.00	7.03	2.50
T_7	65.00	6.70	2.50	16.00	6.84	3.50
T_8	71.60	6.50	2.10	16.00	6.65	4.90
T_9	70.00	6.40	2.10	18.00	6.92	3.90
T_{10}	81.00	6.80	2.20	16.00	6.65	4.90
T_{11}	81.00	6.00	2.60	16.00	6.84	4.10
T_{12}	81.00	6.80	2.20	18.00	6.70	3.50
T_{13}	85.00	6.80	2.10	24.00	6.60	3.70
LSD at 0.05	**1.41**	**0.13**	**0.22**	**1.60**	**0.15**	**0.09**

Table 5. Effect of sodium chloride and acetic acid treatments for 72 and 120 hours on the color and texture of the var. Aseel dates fruit.

Treatments	72 (hours)		120 (hours)	
	color	texture	color	texture
T_1 (control)	Amber color	Less soft, loose	Amber color	Less soft, loose
T_2	Amber color with attraction	Soft, pulpy, loose	Brownish color	Soft, loose
T_3	Brownish brown color but not attractive	Soft, pulpy	Brownish brown color with attraction	Soft, loose
T_4	Amber color	Soft, very loose	Amber and attractive color with shining	Soft, very loose
T_5	Reddish brown color with much attraction	Soft, loose	Brown color with attraction	Soft, loose
T_6	Brown color but not attractive	Soft, pulpy	Dark brown color	Soft, loose
T_7	Brown color but not attractive	Soft, loose	Dark brown color	Soft, loose
T_8	Dark brown color	Soft, loose	Dark brown color	Soft, very loose
T_9	Dark brown color but not shining	Soft, very loose	Dark brown color	very loose
T_{10}	Amber color	Soft, loose	Amber color	Soft, very loose
T_{11}	Amber color	Soft, loose	Amber color	Soft, very loose
T_{12}	Dark brown color	Soft, very loose	Dark brown color	Soft, very loose
T_{13}	Dark brown color	Soft, very loose	Dark brown color	Soft, very loose

Figures

Fig. 1. Var. Aseel tree with fruit at Khalal stage

Fig. 2. Monsoon rains damages the var. Aseel fruit over mats for sun drying

Fig. 3. var. Aseel fruits treated with 13 different treatments of NaCl and acetic acid

Fig. 4. Var. Aseel fruits treated with NaCl showing best accelerating ripening product

The arbuscular mycorrhizal symbiosis and its role in date palm production and sustainability

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ABSTRACT

The arbuscular mycorrhizal association is an essential symbiosis between a unique group of soil fungi and a majority of plant species worldwide. The fungi produce an extensive hyphal network that mines soil for unavailable phosphorus and the plants benefit through improved nutrition. Date palms are shown experimentally to be highly dependent on this association, showing increased trunk and leaf biomass even with applied fertilizers and greater tolerance to saline conditions. This dependency is attributed to a coarse root system with a paucity of root hairs, a high P requirement to support growth of a large plant biomass, and a field environment that further limits P mobility in soil. The fungi require plants to grow yet they have a wide host range, so diverse species can associate with date palms. Many fungal species have considerable genetic plasticity and they are pandemic because of ancient origin and coevolution with their hosts. Experiments show that some nonnative fungal strains can benefit plants as well or better than native strains. Therefore, ecological tolerance permits selection of inoculants that benefit palms in a wide range of environments including those with high salinity. Transport of plants between countries necessitates palm production in soilless media, and use of a light weight mineral-based material supplemented with peat for water-holding capacity will provide an optimum compatibility for the mycorrhizal symbiosis. Many questions remain to be answered, but symbiotic interactions clearly are important in both the production phase and for sustainability after outplanting of date palms to the field.

Key Words: fungi, mycorrhizae, Glomeromycota, mycorrhizal dependency, INVAM

INTRODUCTION

The arbuscular mycorrhizal symbiosis is a critical component to the survival and longevity of a vast number of plant species, including all crop plants (Smith and Read, 1997). A survey of the plant kingdom within the framework of their evolution suggests that all of the earliest land plants formed an arbuscular mycorrhizal association (Trappe, 1987) and that over the intervening eons some plants either evolved different kinds of mycorrhizal associations (of which there are six other types – Smith and Read, 1997) or they lost the symbiosis completely. The latter groups of plants (e.g., Brassiceae, Cruciferae, Chenopodiaceae) tend to be fast growing pioneer species in arid and semi-arid environments that contain few native AMF (Brundrett, 2009). Some of the plant species selected for plant revegetation in desert regions belong to these families and are able to grow in soils lacking these fungi. Other plant species are completely dependent on the mycorrhizal association. In discussing the arbuscular mycorrhizal symbiosis and its potential benefit to date palm production, consideration must be given to the biological and ecological properties of the fungi, the phenology and dependency of plant species serving as host for those fungi, and the environment surrounding both partners. Each of these variables will be considered separately and then applied to date palms.

The fungi

Arbuscular mycorrhizal fungi (AMF) evolved four hundred million years ago at about the same time plants appeared on land. Since those early land plants did not have a true root system for absorption of nutrients, the presence of AMF is hypothesized to have been crucial to evolution of plants on land (Morton, 1990; Pirozynski and Malloch, 1975). AMF can obtain carbon for growth and reproduction only through specialized structures called "arbuscules" that form at a close interface between plant and fungus in root cortical cells (Fig. 1A). As a result, these fungi cannot grow apart from a host plant. Each arbuscule appears to fill a cell, but it resides between the wall and membrane to functionally exchange nutrients between host and fungus. Phosphorus tightly bound to soil particles is absorbed by an extensive network of finely branched hyphae that fills the rhizosphere and it is from these hyphae that asexual spores are formed for widespread dispersal (Fig 1B).

Because this association is an obligate one, the fungi have evolved only with their plant hosts since they appeared on land. For that reason, AMF are unique amongst all fungal groups and this is reflected in their classification as a separate phylum (Schüßler *et al.,* 2001). Co-evolution of AMF with their plant hosts has resulted in some important biological and ecological properties.

Evidence indicates AMF are strictly asexual (Pawlowska, 2005). Spores are produced in soil and roots for dispersal, they germinate, infect the host, grow, and produce more spores. They also are isolated from each other, so that hyphae rarely are able to fuse and exchange cytoplasm and nuclei. Total reliance on asexual reproduction generally is a negative trait because harmful mutations can accumulate, but AMF compensate by possessing many nuclei (Fig. 1C) that move and sort independently throughout the hyphae and into spores (VanKuren *et al.,* 2013). This genetic heterogeneity gives the fungi considerable flexibility and longevity.

AMF species show little evidence of host specificity. This means that any fungal strain can colonize the roots any host plant that is able to form an AM association. Because of this property, a large and diverse international culture collection such as INVAM (Morton *et al.,* 1993; http://invam.wvu.edu) can grow more than 1100 strains of 103 species (44% of total known diversity on one plant host: the highly dependent small grain species, *Sorghum sudanense* (sorghum).

Host specificity and host compatibility must be carefully differentiated, because these terms have been used interchangeably. The former relates to the ability of any fungus to colonize roots and establish a symbiosis. The latter reflects the quality of the symbiosis and plant responses after the association has been established. An unpublished study in INVAM designed to identify the ideal host plants for culturing AMF will serve as an example. Two AMF species with broad ecological tolerance, *Claroideoglomus etunicatum* and *Rhizophagus intraradices*, both were able to colonize apple seedling roots, but only the latter species produced extensive root colonization, sporulated, and caused a strong growth response. When present together, only *R. intraradices* was detected in tree roots after three months of growth. This species was compatible with any plant tested, but *C. etunicatum* was most compatible only with herbaceous plants.

AMF species representing all of the major clades in the phylum Glomeromycota are globally distributed, suggesting that a majority of species are pandemic. This conclusion can be inferred only from phylogenetic patterns, because vast regions of the globe have not been sampled enough. The explanation for such remarkable widespread distribution rests with a combination of evolutionary timing and inherent properties. The fungi are hypothesized to have speciated at a time with the earth's land masses were joined into the supercontinent, Pangea (Morton, 1990). During the next 100 million years, as the continents began to separate, there was ample time and opportunity for asexual fungal spores to disperse and colonize almost any plant growing at the time because of a broad host range. The likelihood of pandemism is important to humans because it means that movement of fungal strains across countries or continents for academic or commercial purposes is safe and not likely to impact on most anthropogenic activities.

Widespread dispersal of species coupled with broad host range results in a high level of species diversity within the root system of even a single plant. From more than 3000 cultures grown at INVAM to trap native AMF species, none consisted of only one species. From more than 3000 cultures grown at INVAM to trap native AMF species, none consisted of only one species. The median number of species was three, 82% were in the range of 2-6 species, and 18% were between 7-12 species. Also, species occupying a root system can represent a broad range of evolutionary clades in Glomeromycota. This range of diversity suggests some AMF fungi have broad ecological compatibility and can occupy many niches comfortably.

Ample evidence exists that AMF species differ in how they benefit plants and the magnitude of that benefit (Brundrett, 1991; Smith and Read, 1997). In some cases, particular matches of AMF and plant species may be needed (Streitwolf-Engel *et al.,* 1997; van der Heijden *et al.,* 1998). A study by Kelly et al. (2005) exemplifies the extent of differential responses among strains of a species as well as between species. Different strains of several AMF species sampled from a range of habitats were compared on a highly dependent warm season grass (broomsedge) in soils containing low to toxic levels of aluminum. All

five strains of one species (*Rhizophagus clarus*) grew well and conferred aluminum tolerance at all Al levels, regardless of the habitat from which they were collected. All five strains of another species (*Acaulospora morrowiae*) failed to grow well, regardless of origin. In contrast, compatibility of *Scutellospora heterogama* varied greatly between strains but those differences didn't correlated with habitat of origin. The broad environmental tolerance of *R. clarus* was revealed as well in the ability of a strain from the Senoran desert in Arizona to grow as well or better in a wetland environment (Fig. 2). Anecdotal data from culturing AMF at different soil P levels in INVAM indicate that *R. clarus* and two other species in *Rhizophagus* (*R. intraradices* and *R. diaphanus*) are able to grow well in plant roots when most other AMF are inhibited completely. It is no surprise, then that *R. intraradices* is one of the most widely and effectively used AMF species in commercial inoculants. *Rhizophagus* species all sporulate prolifically inside plant roots and may be unique in being able to undergo anastomosis, which is the fusion of hyphae to allow exchange of nuclei and cytoplasm (Croll *et al.,* 2009; Purin and Morton, 2013). These properties collectively favor dispersal, longevity, and genetic heterogeneity, making them preferred candidates for broad application as inocula.

The Plant Host

The benefits conferred by AMF to their plant hosts are diverse and governed largely by interactions that optimize mineral nutrition for growth and reproductive potential (or yield in agronomic terms) of the plant host species under consideration. In general, the magnitude of the plant response to the symbiosis is a measure of "mycorrhizal dependency" (Tawaraya, 2011). Fig. 3 illustrates a phosphorus response curve of a perennial cool-season grass that has low dependency and a fruit tree that exhibits complete dependency. The meadow fescue (*Festuca arundinacea*) forms a large biomass of finely branched roots that can exploit immobile P in soil and it does not have a high P requirement for photosynthesis. Fescue is highly dependent on the mycorrhizal symbiosis only when soil P level is extremely low. In contrast, apple (*Malus domestica*) has a very coarse root system and never exploits P availability no matter how high it becomes. Apple fails to respond to any fertility level except when AMF are present. Some plant species with root biomass and architecture similar to that of fescue still are highly dependent on the mycorrhizal association. Warm season grasses have a very high requirement for P because of C4 photosynthesis (Brejda *et al.,* 1993), and a large root system alone cannot compensate.

A typical plant response is an increase in shoot and root biomass, and it is expressed mostly by perennial plant species with strong apical dominance in shoot growth (Brundrett, 1991). However, phenology of the plant also is a major determining factor in the type of response expressed. For example, bulbed plants like bluebells show no top growth response when mycorrhizal, yet they cannot survive in nature without the symbiosis. AMF promote P uptake as expected, but the P is sequestered in the bulbs so that shoot emergence from deep within the soil profile is vigorous (Merryweather and Fitter, 1995).

The Growth Medium

The medium in which any plant is grown determines its nutrition status and its growth potential. When AMF are present, properties and environment of that growth medium can greatly impact on mycorrhizal interactions and the magnitude of any benefit. Many types of media are in use. At INVAM, the standard culture medium for culturing all AMF is a coarse sand and loamy soil mix (3:1 v/v) that is steamed twice to remove native microbes. Sand is used because it is completely inert and increases air spaces for root infiltration. This medium has been used by INVAM for 26 years to culture AMF from all continents and a wide range of habitats, so there is strong evidence for broad compatibility. This mixture is well suited to AMF cultures, but it is not the best option for growing plants commercially that must cross borders between states, countries, or continents.

With the increased emphasis on minimizing movement of pathogens or exotic microbes associated with transport of plant material, soilless media is the only option. Sphagnum peat moss is widely used because of its availability, reasonable cost, light weight, and high water-holding capacity. AMF generally are compatible in peat and peat mixes, despite acidity of the medium.

Expanded volcanic rock material, such as perlite or pumice, are light weight and promote aeration and water retention. Vermiculite has similar properties, but it tends to compress over time and reduce aeration. Expanded calcine clays have gained acceptance in recent years, but pH and nutrient content (especially calcium) varies greatly with where the material was mined and with fertilization regimes. All of these materials provide environments within which AMF are compatible as long as pH stays below 6.2-6.4. One of the key variables, other than pH, is particle size. Ridgeway et al. (2006) show that larger particles promote both root infiltration and mycorrhizal colonization.

Results are most inconsistent with the inclusion of composted pine or hardwood barks. Some studies indicate that AMF tolerate bark media, but tests in INVAM show varying levels of inhibition. For example, two mixes with similar proportions of bark (45%), and peat (55%) produced opposite results. One mix completely inhibited AMF colonization whereas mycorrhizae achieved 46-57% in the other mix. In another test (Fig. 4), two different peat-

hardwood bark formulations were equally inhibitory to mycorrhization, especially in relation to a sand-soil mix. Of the three AMF species examined, one never colonized the corn assay host more than 6% and two other species slowly adapted and colonized at levels as high as 40%. The adaptability and tolerance of the two *Rhizophagus* species corroborate the conclusion reached above that strains of species in this genus have wide ecological tolerance.

Application of AMF to Date Palm Production

Date palm (*Phoenix dactylifera* L.) is naturally colonized by mycorrhizal fungi, based on studies from Iraq (Khudairi, 1969), Saudi Arabia (Khaliel and Abou-Hailah, 1985) and Morocco (Bouamri *et al.,* 2006). The native AMF species present in the rhizosphere can be diverse (Al-Yahya 2008), but at levels similar those in a range of other habitats (Morton *et al.,* 2004).

The dependency of date palm on the mycorrhizal symbiosis is expected to be similar to that of apple (Fig. 3) and other fruit tree species. All are long-lived perennial species with a high demand for nutrients to sustain a large above-ground biomass and fruit production. Moreover, the root systems are coarse with a low degree of branching and root hair formation (Fig. 5). The arid habitat in which date palms are grown contributes even more to mycorrhizal dependency. Phosphorus is mostly immobile and relies on diffusion through a water film from soil particles to root surfaces. In an arid climate, this condition occurs infrequently without human intervention (irrigation).

Few studies have been conducted with the intent of evaluating mycorrhizal dependency of date palm to the mycorrhizal symbiosis. Ghulam Shabbi, Ewald Sieverding and coworkers conducted a year-long experiment to measure mycorrhizal responses in pots of the variety Khaneizi watered with a standard and one-third strength fertilizer and at three salinity levels. While this study did not measure a P response curve for date palm to provide a direct measurement of mycorrhizal dependency, results were dramatic and clearly verified a strong reliance of the mycorrhizal symbiosis (Fig. 6). Predictably, the greatest growth benefit occurred with lower fertility and application of fresh water (Fig. 6A). Most notable, however, was that this benefit occurred even with the standard fertilizer regimen and at increasing salt levels. Amelioration of salt stress by AMF may not be that uncommon, as it has been measured in other plants as well (Al-Karaki, 2000). Fig. 6B shows plant phenotypes of mycorrhizal plants with increasing salinity, and reductions in plant growth are not dramatic.

There are several caveats to this study that accentuate the benefit attributable to the mycorrhizal symbiosis. First, the nonmycorrhizal treatment must have contained a low density of native AMF, as evidenced by fairly extensive colonization after a year's growth in pots. The marked growth benefit that occurred with a symbiosis in all pots indicates that rate of colonization is crucial to the final growth response. Inoculation with *R. intraradices* likely led to rapid mycorrhization and an early growth spurt that became magnified over time, whereas slow AMF colonization in nonmycorrhizal pots took much longer to express a growth benefit. If the nonmycorrhizal treatment lacked any AMF colonization, the differences likely would have been much greater. This result dramatically shows the benefit of inoculation in native environments where AMF may be present but in low density. Second, this experiment was conducted in pots. Roots were constrained over time, thus slowing above-ground biomass in the fastest growing plants. Nutrient levels were more homogeneous and more plant-available (no matter how much was added), so that diffusion of P in the fertilizer would be enhanced by sustained moisture in the pot contents. There was no growth depression (as seen with fescue in Fig. 3) under higher fertility and mycorrhizal colonization in the inoculated pots was reduced, but not appreciably. In nature, none of these conditions would be met, so that P availability would be low no matter how much fertilizer was applied and so mycorrhizal colonization likely would not be inhibited. Collectively, these results suggest that inocula of AMF can improve the production of date palms throughout the Middle East and North Africa and improve crop sustainability after outplanting to the field.

With increased demand and the need for movement of plants across national borders, date palms are increasingly being started from tissue culture and then transplanted into soilless media (Awad 2008). The type of medium used in date palm production, therefore, is of major importance. Based on the discussion above, a recommended medium would consist of mineral component such as expanded rock other than calcined clay (such as a pumice-like material) to keep pH below 7.0 amended with a small amount of peat to optimize water-holding capacity. Barks should be avoided because organic components have a high probability of inhibiting mycorrhization. Other organic material such as compost may be less toxic, but results are likely to be unpredictable. Keep in mind that these fungi evolved in high mineral soils and therefore will be most compatible with their plant host in this environment.

Of the more than 230 AMF species currently known (http://invam.wvu.edu), choice of species and strains of AMF that can serve as the "best" inoculant is dictated primarily by breadth of tolerance to a range of environmental variables. As discussed above, the most likely AMF candidates are in the genus *Rhizophagus*. Because of the pandemic distribution of this and other *Rhizophagus* species (in particular *R. clarus*), their introduction into any field or nursery setting is not

likely to be harmful. Assumptions that native species are the best adapted are not always true. Certain AMF species, such as those in *Rhizophagus*, behave similarly in very divergent environments (e.g. desert, wetland, high P soil, high organic matter, root organ culture, high heavy metals, etc.) and could outperform native strains (see Fig. 2).

The central issue in date palm production is whether inoculation is necessary or not. Results chronicled above provide a strong indication that introducing a quality inoculum into a soilless medium for nursery stock is a necessity for vigorous and sustainable plant growth. This benefit is likely to continue in the field setting. While evidence indicates date palms in the field are mycorrhizal and the fungi are taxonomically diverse, little is known about the density and infectivity of these native AMF communities. Important questions that have yet to answered include the following: What is the inoculum potential of native fungi in the field at the time of date palm outplanting? How much does inoculum potential differ with variation in edaphic conditions (temperature, pH, frequency of rainfall, duration of solarization, etc.), the composition and density of pre-existing plant communities, geographic location, and previous anthropogenic practices or disturbances? How does inoculum potential change with age of date palm groves, especially after onset of fruit production? What is the sustainability of an introduced AMF species (the inoculant) over time as it competes with native AMF? These questions indicate that much has yet to be learned to fully understand and predict the relationship between AMF and date palm, especially after transplanting to the field.

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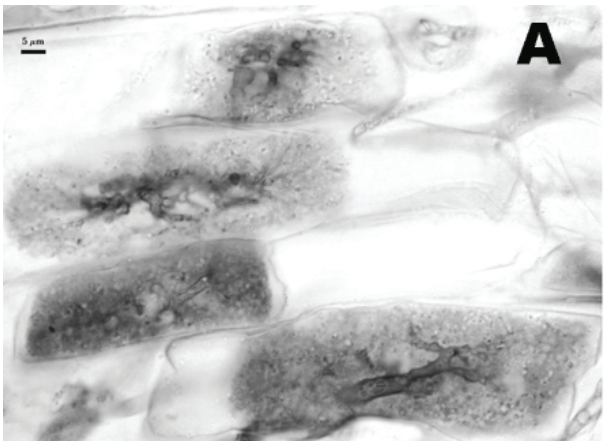
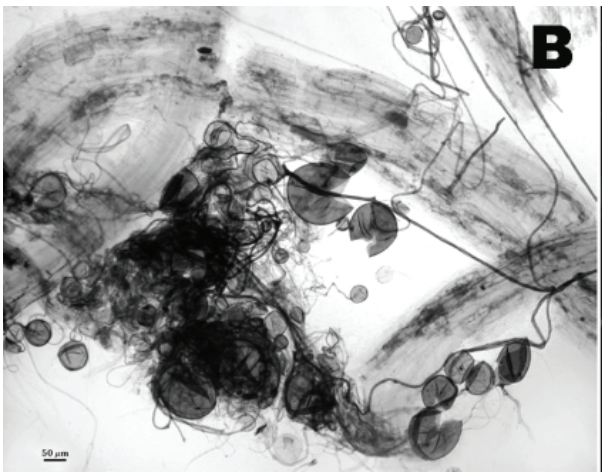
Figures





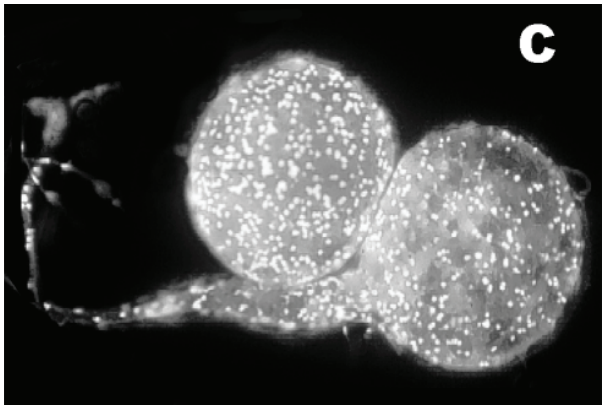


Fig. 1. Basic features of arbuscular mycorrhizal fungi. A. Arbuscules present within the cortical cells of a corn root. B. Mycorrhizal colonization of roots, with external hyphae and asexual spores. C. Multinucleate contents of developing spore and attached hypha.

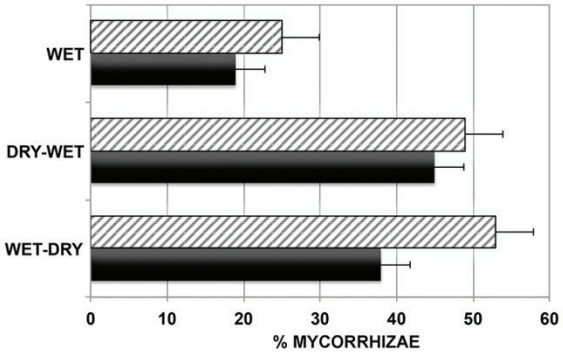


Fig. 2. Mycorrhizal colonization of *Spartina patens* in an artificial wetland by native (black) and non-native strain (hatched) of *Rhizophagus clarus* from the Senoran Desert. Wet = flooded always, dry-wet = mostly dry with short flooded periods, wet-dry = mostly flooded with short dry periods

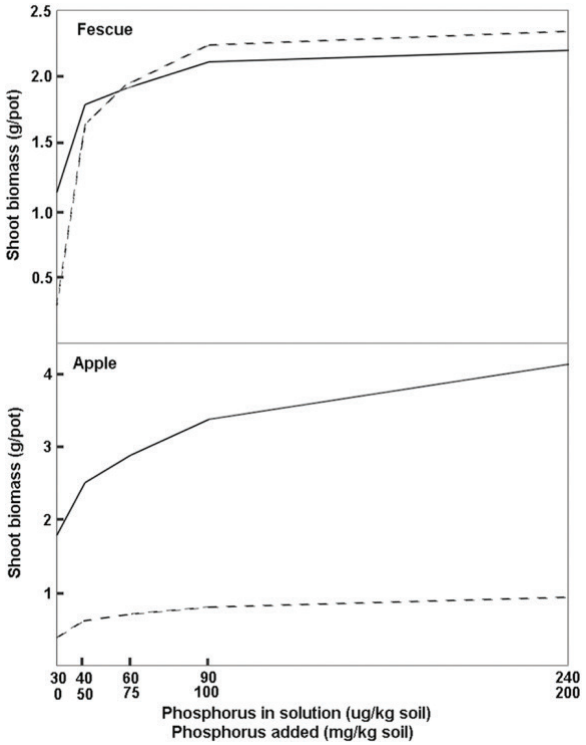


Fig. 3. Mycorrhizal dependency of fescue (*Festuca arundinacea*) and apple (*Malus domestica*) based on phosphorus response curves after 72 days of growth in soil with added P. Solution P was determined by carbon tetrachloride displacement. Dotted line = nonmycorrhizal, solid line = mycorrhizal plants.

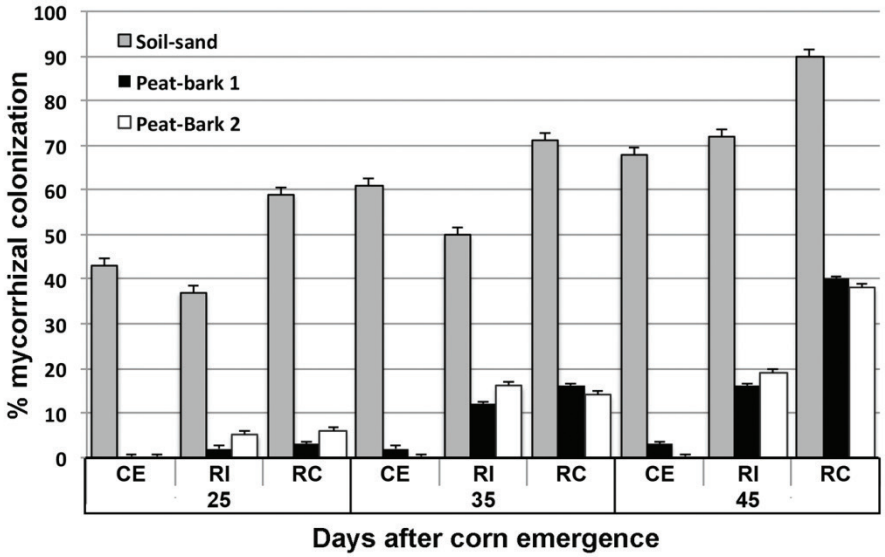


Fig. 4. Variation in amount of mycorrhizal colonization by strains of three AMF species grown on sorghum for 25, 35 and 45 days after emergence. Soil-sand = 1:3 v/v), both peat-barks contained a mix of 40% peat, 45% bark, 15% perlite. CE = *Claroideoglomus etunicatum*, RI = *Rhizophagus intraradices*, RC = *Rhizophagus clarus*,

Fig. 5. Root mass and architecture of mycorrhizal and nonmycorrhizal date palm, variety Khaneizi.

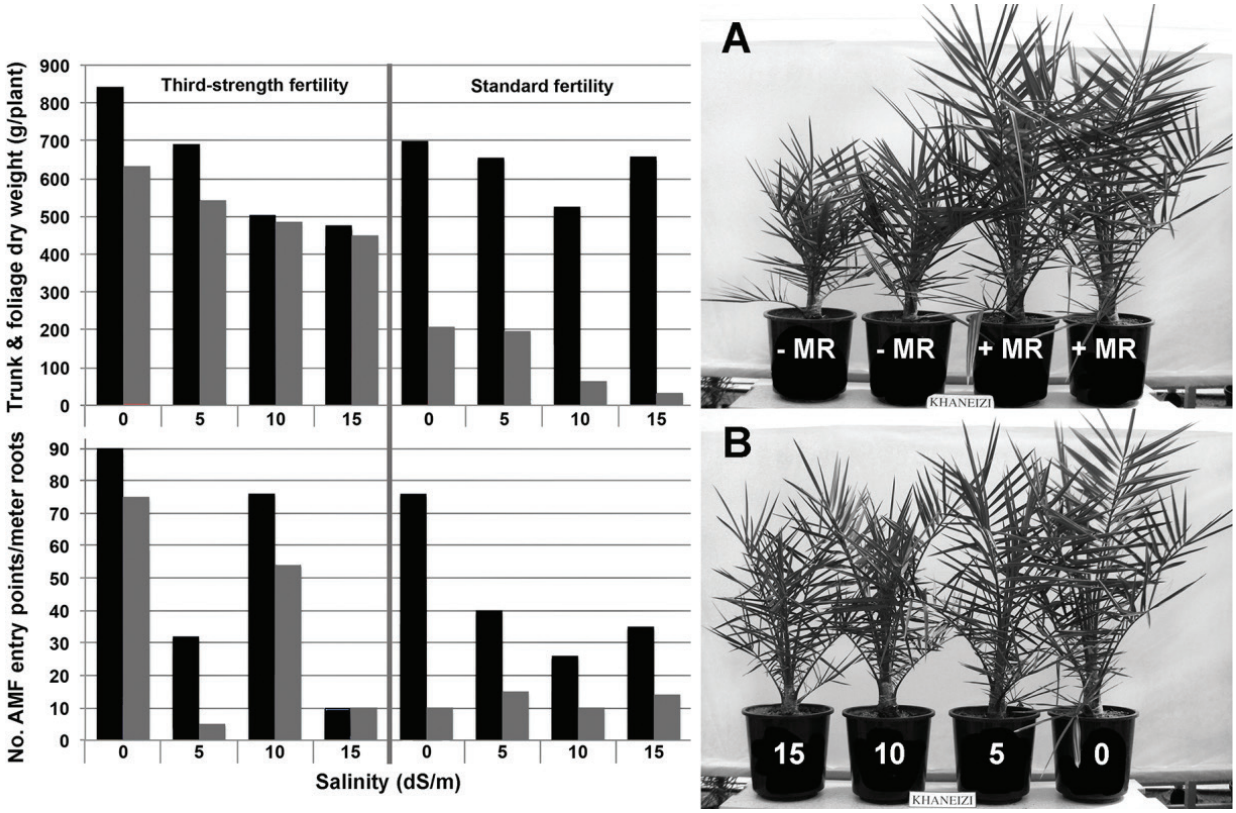


Fig. 6. Reponses by one-year-old date palms, cultivar Khaneizi, to inoculation with *Rhizophagus intraradices* and watered at varying levels of salinity (0-15 dS/m). Plants were grown in a medium consisting of native soil 1.5 m below root zones and a mix of peat and perlite (2:1 v/v). In the chart, blue bars represent mycorrhizal plants and the red bar indicates nonmycorrhizal plants. MR = mycorrhiza. A = paired plants at one-third strength fertility with or without AMF added. B = Plants at standard strength fertility inoculated with AMF and watered at three salt concentrations.

Seasonal incidence and abundance of palm borers in date palm plantations of Al-Hassa based on light trap captures

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ABSTRACT

The Al-Hassa region in the Eastern Province of Saudi Arabia, with an estimated three million date palms is the Kingdom's premier date palm oasis. Besides the red palm weevil, Rhynchophorus ferrugineus a group of three Coleopteran beetles viz. the stalk and stem borer, Oryctes spp Prell (Coleolpera : Scarabaeidae) the long horn stem borer, Jebusea hammerschmidti Reich (Coleoptera : Cerambycidae) and the frond borer Phonapate frontalis F. (Coleoptera : Bostrichidae) are wide spread in the Al-Hassa oasis and are emerging as major insect pests of date palm in the region. Adults of these Coleopteran beetles are known to be attracted to light traps. We studied the seasonal incidence and abundance of these beetles using 18 Robinson light traps stationed in the northern, central and southern villages of Al-Hassa for two years during 2010 and 2011. Observations were recorded once a week on the captures of these beetles in the traps. Results on the seasonal incidence indicated that, J. hammerschmidti was most active during May, June and July while Oryctes spp recorded maximum activity during June and July. Further, peak activity of P. frontalis was recorded during April and May. J. hammerschmidti was most abundant in the northern villages of Juleijlah, Muteirfy and Ain Mansour while it was least prevalent in the the south of the oasis in Al-Gowaybah. Oryctes spp was recorded in all the study areas and was most prevalent in Batalyah, Ashura and Kilabiyah in the centre of the oasis. Among the three beetles studied, P.frontalis was the least prevalent and could be considered of minor importance. There is a need to develop an Integrated Pest Management (IPM) strategy to combat the increasing incidence of these Coleopteran beetles in date palm.

Key words: Date Palm, borers, seasonal incidence, abundance

INTRODUCTION

The Kingdom of Saudi Arabia is among the top three date producing countries of the world with an annual production of over a million tones of dates accounting for 17% of the global production (http://faostat.fao.org). Saudi Arabia is estimated to have 25 million date palms with more than 400 different date palm cultivars (Anonymous 2006).The Al-Hassa region in the Eastern Province of Saudi Arabia, has three million date palms and is the Kingdom's premier date palm oasis (El-Baker, 1952; Asif *et al.,* 1982).

Coleopteran beetles have gained importance during the last 2-3 decades among which red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera:

Curculionidae), is a key pest of date palm, first recorded in Al-Hassa during 1992 (Faleiro *et al.,* 2010; Anonymous, 1998). El –Sabea *et.al*., 2009 estimated the annual loss due to eradication of severely infested palms by RPW in Saudi Arabia to range from USD 1.74 to 8.69 million at an eradication level of 20 percent infested palms, at 1-5 per cent infestation, respectively. Besides RPW, a group of three other Coleopteran beetles *viz*. the stalk and stem borer, *Oryctes* spp Prell (Coleolpera : Scarabaeidae) the long horn stem borer, *Jebusea hammerschmidti* Reich (Coleoptera : Cerambycidae) and the frond borer *Phonapate frontalis* F. (Coleoptera : Bostrichidae) are wide spread in the Al-Hassa oasis and are emerging as major insect pests of date palm in the Gulf region (Aldryhim, 2008; Al-Deeb,2012; El-Shafie, 2012). These pests bore into the trunk, fruit stalks or leaf fronds resulting in loss of yield or even death of the palm incase of severe infestation by the stalk and stem borer and also the long horn stem borer.

Adults of these Coleopteran beetles are known to be attracted to light traps (Khalaf *et al.,* 2012; Al-Deeb, 2012). We studied the seasonal incidence and abundance of these beetles using 18 Robinson light traps stationed in the northern, central and southern villages of Al-Hassa for two years during 2010 and 2011.

MATERIALS AND METHODS

18 Robinson light traps were set in date palm plantations in the northern, central and southern villages of the Al-Hassa oasis (25^{O} 19’ 60” N latitude and 49^{O} 37’ 60” E longitude) during late 2009 as indicated below.

In the north, six traps were set the villages of Oun, Murrah, Juleijlah, Muteirfy and Ain Mansour. In the centre of Al-Hassa, nine light traps were set in Omran, Taraf, Al-Jisha, Jubail, Mizawi, Muneizlah, Batalyah, Ashura and Kilabiyah while in the south three Robinson light traps were set in the village of Al-Gowaybah.

Weekly observations were recorded on the adult beetle captures of the stalk and stem borer, *Oryctes* spp, the long horn stem borer, *Jebusea hammerschmidti* and the frond borer *Phonapate frontalis* for the entire duration of 2010 and 2011. Trap capture data was tabulated every month during the study period.

Monthly mean data including standard error of means for the beetle capture with respect to the seasonal incidence and abundance of the above three Coleopteran beetles was calculated using the web-based agriculture statistics package (WASP.1) available at www.icargoa.res.in

RESULTS AND DISCUSSION

Seasonal incidence

Results presented in figure 1 indicate that *Oryctes* spp and *J.hammerschmidti* attained peak activity in June during the summer. While *Oryctes* spp was active from March to November, *J.hammerschmidti* was active from March to August. Our findings are in agreement with a previous report from Al-Hassa which indicates highest adult activity *Oryctes elegans* and *J.hammerschmidti* during the summer months of July and June, respectively (Hammad, *et al.,* 1986). Studies conducted in Iraq indicate that *Oryctes elegans* is active from April to December attaining a peak during the summer in July.

The monthly mean *Oryctes* spp and *J. hammerschmidti* was 3.14±2.03 and 0.56±0.19, respectively. Aldryhim, 2008 reported that *J.hammerschmidti* was most active in Saudi Arabia during the months of May and June. With regard to number of generations per year, the study indicates that both *Oryctes* spp and *J.hammerschmidti* had one generation per year with single population peaks and is in agreement with previous reports by Najeej *et al.,* 1993a,b; Al-Deeb 2012.

In our study we recorded two species of the stalk and stem borer. However, previous reports from the region indicate the possibility of three species *viz* . *Oryctes elegans, O.agamemon and O. rhinoceros* to exist in the date palm plantations of the Middle East. One of the species endemic to the greater Middle Eastern region is *Oryctes agamemnon* (Coleoptera: Scarabaeidae) (Gassouma, 1991; Howarth and Gillett, 2008; Soltani *et al.,* 2008,). Two other species, *Oryctes rhinoceros* and *Oryctes elegans*, have been found in the region, although their distribution and exact impact on date palm plantations are not known (Gassouma,1991). Studies using pheromone lures will help ascertain the species complex of the stalk and stem borer in date palm.

Studies carried out in the UAE also indicate that *O.agamemon* is a univoltine pest with a single population peak. Adults appeared in the field around middle of April and early May and the population continued to build until maximum numbers were reached in mid June. No adults were found after the end of September. Photoperiod showed a significant correlation with the changes in adult population size (Al-Deeb *et. al*., 2012).

With regard to the frond borer, figure 1 reveals that this beetle was active from March to November with two peaks during April and November. The monthly mean for *P. frontalis* was 0.56±0.19. A report from Iraq indicate activity of this pest from April to December (Khalaf *et al.,* 2012).

Population abundance

Figures 2, 3 and 4 indicate the density of *Oryctes* spp*, J. hammerschmidti* and *P. frontalis* in the north, centre and south of the Al-Hassa date palm oasis. While *J. hammerschmidti* and *Oryctes* spp were dominant in the north and centre of the Al-Hassa oasis, *P. frontalis* was found to be important in the south of Al-Hassa. This can be attributed to the fact that both *J. hammerschmidti* and *Oryctes* spp are known to attack older date palms (Aldryhim, 2008, Al-Deeb, 2012) which exist in the north and centre of the oasis. *J. hammerschmidti* was most abundant in the northern villages of Juleijlah, Muteirfy and Ain Mansour while it was least prevalent in the south of the oasis in Al-Gowaybah. *Oryctes* spp was recorded in all the study areas and was most prevalent in Batalyah, Ashura and Kilabiyah in the centre of the oasis. Most of the date plantations in the south (Al-Gowaybah) of the Al-Hassa oasis are comparatively young (about 15-15years old) suitable for the frond borer, *P. frontalis*.

Of the three palm borers studied, *Oryctes* spp had the highest density and was most abundant followed by *J. hammerschmidti*. Based on our findings, these two palm borers could be considered as major pests of date palm in Al-Hassa as also indicated previously by Aldryhim, 2008 and Al-Deeb *et al.,* 2012 for which integrated pest management (IPM) strategies have to be devised. Based on our findings the frond borer, *P. frontalis* could be considered of minor importance.

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Figures

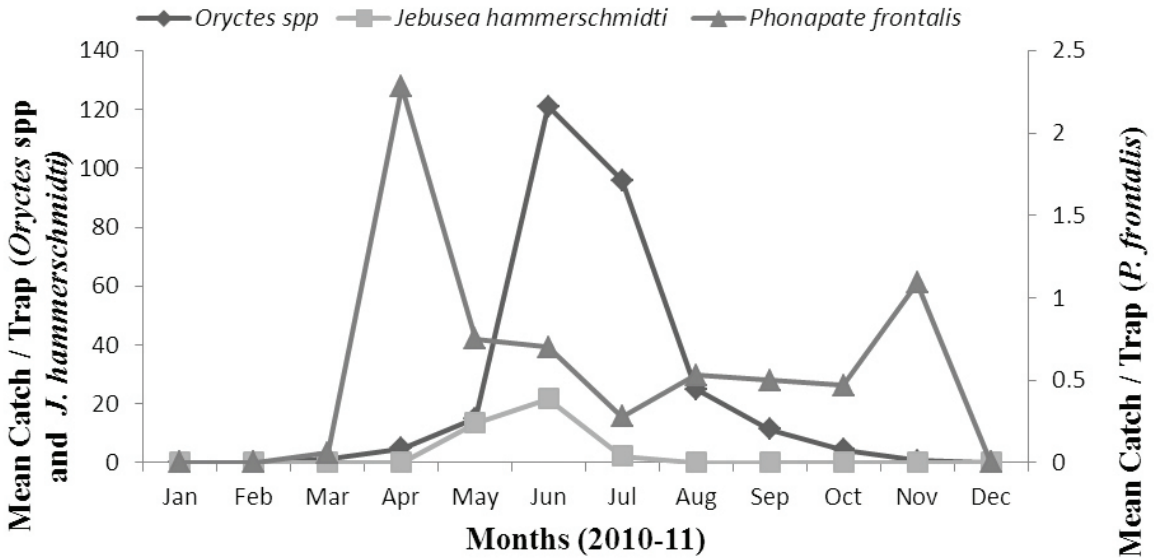

Figure 1. Seasonal incidence of palm borers attracted to light traps in date palm (Al-Hassa, Saudi Arabia, 2010-11). The monthly mean along with standard errors (±SE) for *Oryctes* spp, *J. hammerschmidti* and *P. frontalis* are 23.30± 11.79, 3.14±2.03 and 0.56±0.19, respectively

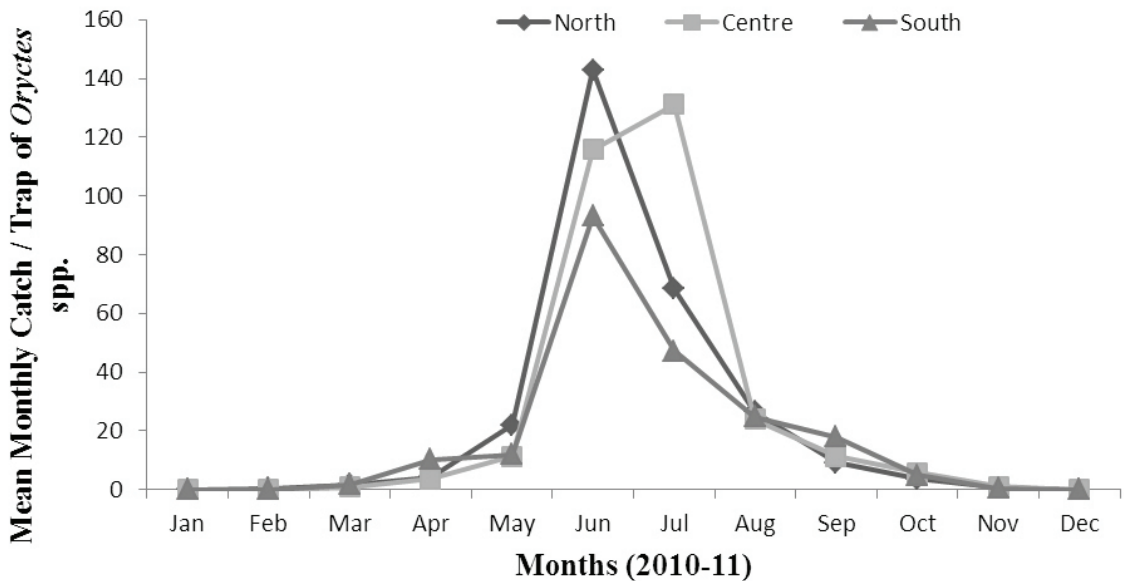

Figure 2. Abundance of *Oryctes* spp in date plantations of Al-Hassa, Saudi Arabia (2010-11). The monthly mean values for the north, centre and south of the oasis are 23.31±12.27, 25.39±13.41 and 17.68±7.96, respectively

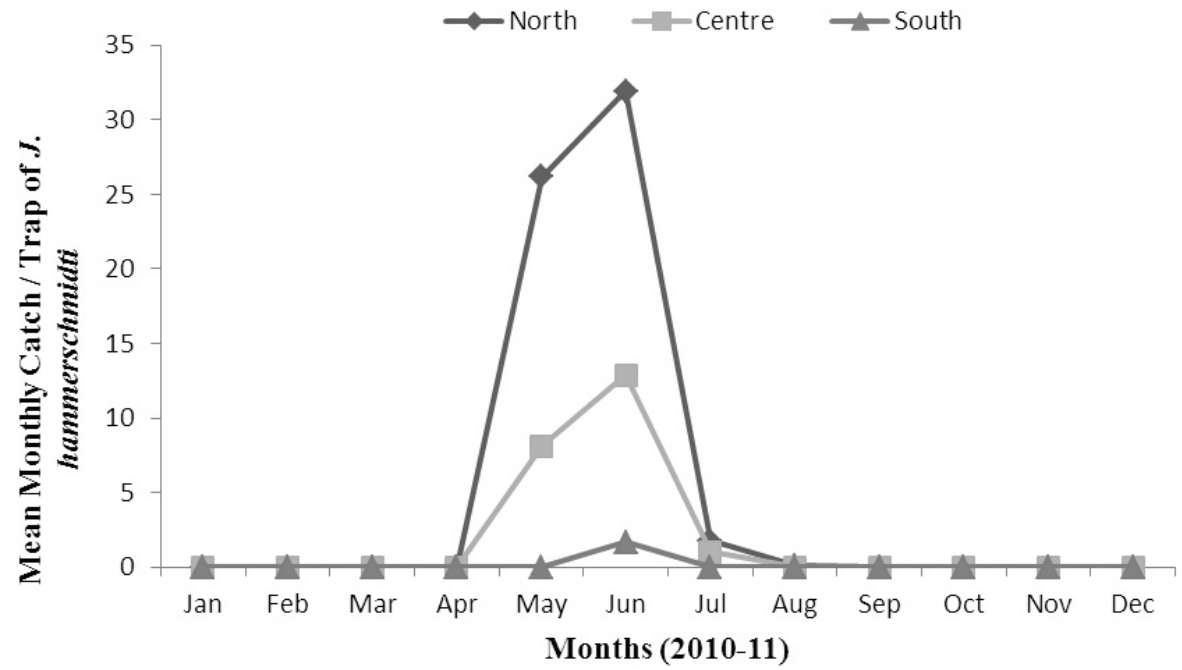

Figure 3. Abundance of *Jebusea hammerschmidti* in date plantations of Al-Hassa, Saudi Arabia (2010-11). The monthly mean values for the north, centre and south of the oasis are 5.00±3.27, 1.83±1.21 and 0.14±0.14, respectively

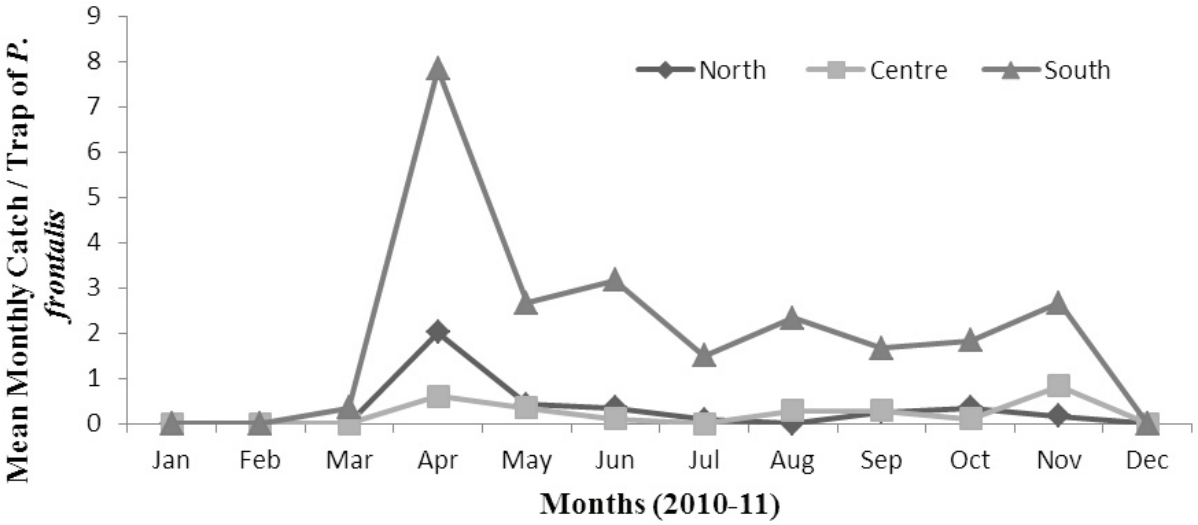

Figure 4. Abundance of *Phonapate frontalis* in date plantations of Al-Hassa, Saudi Arabia (2010-11). The monthly mean values for the north, centre and south of the oasis are 0.30±0.16, 0.21±0.08 and 2.00±0.63, respectively

Field Efficacy of bio-rational pesticide fytomax N against dubas bug *Ommatissus lybicus* De berg (Homoptera: Tropiduchidae) in autumn and spring generation

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ABSTRACT

Dubas bug represent the most economic important pest on date palm in Yemen, especially in eastern coastal area(Coast of Hadramout, Shabwa and Al-Mahra). National campaigns carried out annually by using ground application of conventional chemical pesticides include pyrethriod and organophosphate. Many of which are effective but simultaneously it kill beneficial insects like parasite, predator and bees. Even though Dubas bug can be adequately controlled with chemical insecticides,but the cost availability, health and environmental risks impose serious limitations on the use of conventional chemicals. Recently Dubas bug has also acquired reduction in susceptibility to insecticides that have been previously effective. Therefore, an alternative approach is urgently needed for controlling this devastating pest. One possible solution is the use of natural bio-rational plant extract that could be safe, sustainable, eco-friendly and effective control measure and to achieve this approach, field efficacy by using commercial neem insecticides. for this purpose Fytomax N(Azadirachtin 1%) has been selected for dubas bug control in organic date palm cultivation in 2012-2013. Fytomax N was applied at a rate of 3ml/litre of water in the spring using HV sprayer in the area of Ghaidat Albhich in May 11,2013 and in autumn-generation in Valley Asd Aljabel at coast of Hadramout on October22,2013 in the area of severely infested palms on which no pesticides have been used for several years. The dominant individuals were fourth nymphal instar and adult stages present in the experimental site. Comparisons have been made with Dimethoate 40 EC in the autumn generation, while Desirin 250EC(Deltamethrin) at spring generation at a rate of 1ml/ litre water and untreated control. The results have been statistically analyzed and revealed control of nymphs and adult was(86,87%),(89.8,87%) and (89.8,86.5%) respectively, in spring generation after one day, one week and two weeks after application of Fytomax N, While Decirin 250 EC Efficacy was an average of 98.5%. In autumn generation the dubas population was less in treatment area in compare to the untreated control plots. The efficacy of Fytomax N on controlling nymphs and adult stages was (92.42, 94.0%) and (94.7,93.74%) on week and two weeks after application respectively, moreover, no significant deference was observed on dubas bug population decline while applied of Fytomax N and Dimethoate in the treated area. This outstanding performance encouraged

us to recommend the inclusion of Fytomax N in Dubas bug control National campaign in Yemen as a green bio-rational solution. Future work will focus on biological aspects of Fytomax N on eggs lying,eggs hatching of dubas and its impacton the egg parasitoid Pseudoligosita babylonica.

Key words: Dubas Bug, Fytomax N, Field Efficacy. Date palm, Hadramout, Yemen.

INTRODUTION

Dubas bug represent first economic importance in many Arab countries and in Yemen. especially in the eastern coastal area (the coast of Hadramout, Shabwa and Al-Mahra), where the Dubas insect cause significant economic damage, which call for the use of large amounts of chemical pesticides (Al-Baker, 1972; Abdul Hussain 0.1974; Al-Jboory1999; Bashmilah.2002; Alshamsy, 2002; Baangood and others, 2009). Health and environmental problems that accompanied the use of chemical pesticides, as well as the economic costs of these pesticides has stimulated researchers to use Botanical, organic plant-based pesticides (Jurani .1991, Al-Rubaie and others, 1992 and Zidane, 2002) which can be a safe alternative for chemical pesticides manufactured currently in circulation because it has desirable specifications which is not available in chemical pesticides, including rapid analyzes to non-toxic materials as a result of their sensitivity to intense light, heat and humidity and it›s low toxicity to humans, animals and non-target organisms (Al-Jboory and others, 1999, and Ashamsy 2002),and non-toxic to plants in recommended dose, and it can be manufactured locally (Al-Rubaie and others, 2000) moreover it can not be considered as environmental pollutants (Kleeberg and Hummel2001) . The difficulty of appearance of pests resistance, against these pesticides is over come ((Mitchell, 1990), in addition to the weakness of their effect on the parasites eggs and the Predators (Raguraman and Singh, 1999 and Akol et.al., 2002 and Simmondset.al.,2000 and Schmutterer,1997).

The plants represent the richest sources of bioactive chemicals, where there are around 2,400 plant contain chemicals with the effectiveness of the lethal lesions are distributed in 189 various plant families (Grainge and Ahmed, 1988). Plants species belonging to the *Meliaceae* family represent the most important plant families in this aspect due to the large number of species of adverse effects on pests and especially *Azadirachta indica* and *Melia azedarach* L. (Singh and Wahab, 1996.)

It is found that Salanin and Azadirachtin chemicals compounds, that have been isolated in a pure form, from Neem and *Melia azedarach* L, have a good effectiveness on several pest insects(Warthen,1979). Al-Rubai and others.2000, mentioned that aqueous and oil extracts influenced to kill nymphs and adults of Dubas at laboratory .however field efficacy of neem aqueous extracts proved a good effectiveness on nymphs and adults of dubas bug(Bashomaila,2011),and on pests like leaf miner on citrus, stem borer on Tabacco, *Earias insulana* on Okra and Thrips on onion(Bashomaila,2006). The neem tree in one of these plants scattered in Hadramout region have several uses (Bashomaila and Bamossa,2012). commercial formulations of Neem have shown high field effect on various stages of dubas bug as noted by Al-Dhamen 2002. Spraying campaigns by using commercial preparations of Neem in Iraq have been effective according to the Iraqi -Date palm website(2011).

Depending on the data above and on our experiences preliminary earlier in this context field efficacy of Fytomax N pesticide prepared from neem oil has been conducting, which provided to us thankfully by British company Russell IPM, to be used in biological control of Dubas bug, hoping to be included in the integrated management in organic farming of date palm.

MATERIALS AND METHODS

To test the effectiveness of the neem pesticide Fytomax N which contains the active ingredient Azadrechtin 1% (10,000 ppm) and neem oil 30% made by private technology company Russell to fit fumigation and ground spraying. One of the infected fields was selected in autumn generation in the area of Valley Asd Al-Jabel in the October 11, 2012 and in the spring generation in the Ghaidat Albhich Valley on May 11, 2013 at the fourth nymphs age and adult of Dubas bug, where the infestation is present on most of the trees with the presence of honey. The experiment was conducted in four areas where each treatment area ranged about half an acre (2100m^2) from each treatment five tree palms were chosen, the data and analyzed based on randomized complete block design in five replicates.

Inspection was conducted before spraying and one day, one week and two weeks after spraying, by inspecting each of the four fronds in different directions, ten leaflets of each frond where inspected, by calculating nymphs and adult insects. Attributed to the effectiveness of the control numbers of nymphs and adult before spraying and after spraying was measured by the following equation:

$$\text{Effectiveness\%} = \frac{\text{insect number after spraying} - \text{Insect number before spraying}}{\text{Insect number before spraying}} \times 100$$

Spraying each treatment was conducted by using the sprayer Capacity thousand liters of high-pressure machine ad also100-liter machine was used when necessary. Packed the machine with water and then add the necessary dose of experience materials as the table below:

No.	Formulation	Active ingredient	Dosage
1	Fytomax N	Azadirachtin	3ml/l water
2	Dimathoate	Dimathoate	1ml/l water
3	Disirin	Deltamethrin	1ml/l water
4	by water Spraying	Tap water	10ml/ palm tree
5	Control		

Data were subjected to statistical analyze using Costat program.

RESULTS AND DISCUSION

Fytomax N effectiveness in autumn generation:

Table.1 describes the result obtained from this experiment in autumn generation, howed that, the natural pesticide Fytomax N one week and two weeks after application, had a clear effect on insect numbers of Dubas bug (nymphs and adult), which decreased to (2.06, 0.32) and (1.56,0.3) insect/leaflet respectively, with significant difference for the treatment of water spray, which reached (18.12,4.24) and (20.12,3.22) respectively, as well as with the control treatment, which had increased the Dubas bug population to (26.56,5.34) and (29.92,4.6) respectively, moreover, no significant deference was observed on dubas bug population decline while applied of Fytomax N and Dimathoate in the treated area, except population of adult two weeks after spraying.

It is also notes from the same table that, the treatment of spraying by water has reduced the nymphs population significantly compared to the control a week and two weeks after spraying, however there is no significant decline of adults compared. to the control.

Results in table2 showed a big reduction of Dubas bug population a week and two weeks after Fytomax N application revealed good control of nymphs and adult Was (92.24%,94.0%) and(94.7%,93.5%) respectively, moreover it is noticed that, the death percentage was high for the treatment of Fytomax N, and more than that of Dimathoate insecticide, which effectiveness had decreased rapidly two weeks after application to 87.83% and 75.21% respectively(tab.2).

The effectiveness of the pesticide in the spring generation

It is clear from the results of Table (3) that the spraying by Fytomax N pesticide in spring generation gave similar results with the results of the spraying in autumn generation, but at lower rates, which decreased Dubas population significantly compared to the control, but this decline did not differ significantly with Disiri treatment a day, one week and two weeks after application and with the treatment of mixture of Fytomax N and Disirin a week and two weeks after spraying, which has reflected on the effectiveness against nymphs and adults. Effectiveness of the FytomaxN a day and one week two weeks after spraying (85.9% and 87%) and (89.8% and 86.5%) and (89.8 and 86.5%, respectively) (4)..

Effectiveness of Disirin insecticide a day after spraying on the nymphs and adult was (97.9% and 98.3%) while that the one week and two weeks after spraying was (97.3% and 94.5%). The mixture of Fytomax and Disirin Gave greater efficiency a week and two weeks after spraying it was (98.9% and 97.8%) (Table 4).It can be observed that the rate of death percent and effectiveness of the pesticide Fytomax N on nymphs was higher than on the adult, and this is agree with results found by Al-Rawi and Hamidawi (1999) who mentioned that the adult insect of Dubas bug is more tolerant than nymphs stage.

The obtained results in this research, in which commercial neem biorational pesticide Fytomax N with concentration of 1% Azaderachtin is used refers to high rates of death for nymphs and adults of Dubas bug in two generations, these are agree with what indicated by Al-Dhamen (2002) who notes that, the concentrations of (0.5-3%) of the commercial neem pesticide Superneemic revealed to high death rates of fourth instar of nymphs ranged from 66.66 -100% and 63.3-100% for two generations, while on fifth stage ranged from 60-100% and 57-100% for two generations., however percentages of adult death ranged from 50.3 to 97% and 47-100% for two generations. Moreover this formulation had a repellent effect on Dubas bug.

RECCOMMENDATION

The experiment concluded that the effectiveness of the Natural Neem pesticide Fytomax N reached in the spring generation (89.8% and 86.5%) on fourth nymphs age and adult respectively however, were higher in the autumn generation and reached (94.7 and 93.74%) on nymphs and adult respectively.

This outstanding performance encouraged us to recommend the inclusion of Fytomax N in Dubas bug control in National campaign in Yemen as a green bio-rational solution. Future work will focus on biological aspects of Fytomax N on eggs lying, eggs hatching of dubas and its impact on the egg parasitoid *Pseudoligosita babylonica.*

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Tables

Table 1. Number of Dubas Bugs after control in autumn season of 2012

№	Treatments	7DAT		14 DAT	
		Nymphs	Adult	Nymphs	Adult
1	Fytomax N	2.06c	0.32c	1.56c	0.30b
2	Dimathoate	1.84c	0.58c	3.64c	1.14c
3	Water spray	18.12b	4.24b	20.12b	3.22a
4	Control	26.56a	5.34b	29.92a	4.60a

Table 2. Efficacy of Fytomax N for controlling Dubas in autumn season 2012

№	Treatments	7DAT		14 DAT	
		Nymphs	Adult	Nymphs	Adult
1	Fytomax N	92.24	94	94.7	93.5
2	Dimathoate	93.07	89.13	87.83	75.21
3	Water spray	31.77	20.59	32.75	30
4	Control	----------	---------	----------	---------

Table 3. Number of Dubas bug after control with Fytomax N in the Spring season 2013

Treatments	BT		1DAT		7DAT		14 DAT	
	Nymphs	Adult	Nymphs	Adult	Nymphs	Adult	Nymphs	Adult
Control	12	15.3	19.20a	17.70a	11.16a	5.54a	8.70a	4.54a
Fytomax N	15.6	14.3	2.70b	2.34b	1.14b	1.30b	0.85b	0.92b
]Disirin	26.6	22.3	0.88b	0.30b	0.30b	0.18b	0.18b	0.18b
Fytomax N+Disirin	18.07	17.3	-	-	0.12b	0.12b	0.12b	0.12b

Table 4. Efficacy of Fytomax N for controlling Dubas bug in Spring generation 2013

Treatments	1DAT		7DAT		14 DAT	
	Nymphs	Adult	Nymphs	Adult	Nymphs	Adult
Fytomax N	85.9	87	89.8	86.5	89.8	86.5
]Disirin	97.9	98.3	97.3	94.5	97.3	94.5
Fytomax N+Disirin	-	-	98.9	97.8	98.9	97.8

Efficiency of some natural enemies against Ephestia cautella walk

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ABSTRACT

Many insect pests attacking date fruits, among the most important of these insect pests Ephestia cautella which attacks stored dates causing great loss, therefore laboratory studies were carried out to evaluate the role of some natural enemies against eggs and larvae of E.cautella. Data show that release of Chrysoperla carnea larvae on E.cautella eggs reduced the percent of emerged adults to 99.2 %, release of Trichogramma evanscens on E.cautella eggs reduced the percent of emerged adults to 98.6% and release of Bracon hebetor on E.cautella larvae reduced the percent of emerged adults to 98%

Key words: Dates - *Ephestia* – natural enemies.

INTRODUCTION

From the estimated 120 million date palms in the world, over two-thirds are in Arab countries (FAO, 1982). Arab countries possess 70% of the 120 million world's date palms and are responsible for 67% of the global date production. Unfortunately, the date palms grown in the Arab region are under threat of diseases, pests, environmental changes and socio-economic factors. Date palm trees declined in the traditional growing areas. As much as 30% of production can potentially be lost as a result of pests and diseases. Almond moth, *Ephestia cautella* (Walker) is a major pest of stored dates {In Egypt, Gough (1917), Hammad et al. (1966)}, In Iraq, Hussain and Jafar (1966), { In Egypt, Saleh (1974), Abdel Salam and El-Saeady (1982), Ali et al. (2003),and Metwalley et al. (2007)} . Natural enemies has an important roles in supressing this pest, predator, *Chrysoperla carnea* (Steph.) larvae against *Ephestia* eggs, The external larval parasitoid *Bracon hebetor* Fay. (Hymenoptera, Braconidae), against *Ephestia* larvae { Hammad et al. (1982), Cline *et al.,*(1984); Gul and Gulel,(1995), Darwish *et al.,*(2003) and Hameed et al (2010))}and Parasitoid *Trichogramma evanescens* Westwood (Hymenoptera:Trichogrammatidae) against *Ephestia* eggs.{ Lewis and Redlinger (1969), Brower (1983), Brower (1984) Bakri (2008).

The objective of this work is studying the effect and efficiency of Chrysoperla carnea Stephens larvae against Ephestia cautella eggs. Moreover studying the effect and efficiency of Trichogramma evanescens against Ephestia cautella eggs and study the effect and efficiency of Bracon hebetor against Ephestia cautella larvae.

MATERIALS AND METHODS

1 - Efficiency of Ch. carnea Staph. Larvae against E.cautella eggs

- Rearing of Ch. carnea were carried out at the Chrysopa Mass Rearing Unit, Faculty of Agriculture, Cairo University. Culture of *Ch. carnea* larvae were reared on *E. kuehnilla* eggs.
- Dates kept under freeze conditions (Gharib and El-Lakwah, 2007) for two days to kill any insect stages inside dates, then it was put in the plastic jars (30 dates/ jar) under constant laboratory conditions. (Temperature 25±2 degree centigrade & 65% ± 5 R.H.).
- The dates were artificially infested by fresh eggs of *Ephestia cautella* (1000 eggs/jar),
- 10 larvae (2nd larval instar) of *Ch. Carenea* were released in each jar except the control. Each jar was covered with muslin. The experiment consisted of 3 replicates.
- Observations recorded on behaviour and development of *Ch. carnea* larvae and numbers of emerged *E. cautella* adults were recorded. Emergence

percentage (*E. cautella*) and reduction% by (*Ch. carenea*) were determined by using Abbott's formula (Abbott, 1925) to correct mortality%. (Mahfouz and Abou Abou El-Ela, 2011).

$$\text{Mortality\%} = \left(\frac{P - P0}{100 - P0} \right) \times 100$$

Where: P = the mortality per cent of treatment, P0 = the mortality per cent of control.

2- Efficiency of T. evanescens against E. cautella eggs

- Rearing of T. evanescens were carried out at the Chrysopa Mass Rearing Unit, Faculty of Agriculture, Cairo University. Culture of T. evanescens were reared under laboratory conditions on E.kuehnilla eggs.
- Dates (semi dry) were kept under freeze conditions for two days to kill any insect stages inside dates, then it was placed in four plastic jars. Dates artificially infested by E. cautella fresh eggs (1000 eggs/jar) under laboratory conditions. (Temperature 25±2 degree centigrade & 65% ± 5 R.H.)
- The cards (1x1 cm) of T. evancses were put in each jar except the control.
- Each jar was covered with muslin for prevents any parasites from entering. The experiment consisted of three replicates. Observations were recorded on behaviour; development and emergence of the T.evanescens by examining E. cautella eggs under binocular to observe black colour of the holes on the eggs caused by T. evanescens pupae. Newly emerged E. cautella adults were also recorded. Emergence percentage of E. cautella, reduction % by T. evancses and emergence percentage of T. evancses were determined.
- Abbott's formula was used to correct mortality % according to Mahfouz and Abou El-Ela (2011).

3 - Efficiency of B. hebetor against E. cautella larvae

- Bracon hebetor adult were obtained from Chrysopa Mass Rearing Unit, Faculty of Agriculture. Cairo University.
- Dates kept under freeze conditions for two days to kill any insect stages inside dates, and then it was placed in four plastic jars (30 dates / jar).
- The dates artificially infested by E. cautella 1st larval instar (50 Larva / jar) under laboratory conditions. (Temperature 25±2 degree centigrade & 65% ± 5 R.H.)
- After fifteen days, one newly emerging pair of Bracon hebetor was placed in each jar with honey droplets (except the control) and covered with muslin. The experiment consisted of three replicates.
- Observations were recorded on behaviour and development of the B. hebetor. Also, newly emerging E. cautella adults were recorded. Percentage of emergence of E. cautella, reduction % and percentage of emergence of B. hebetor were determined. Abbott's formula was used to correct mortality% (Mahfouz and Abou El-Ela, 2011).

RESULTS & DISCUSSION

1- Efficiency of Ch. carnea Staph. Larvae against E. cautella eggs

Data in Table (1) revealed the efficiency of Ch. carnea larvae against E. cautella eggs. After 30 to 45 days from artificial infested dates by E. cautella eggs., the percentage of adult emergence of E. cautella ranged from 0.1 to 0.2% with an average of 0.13±0.06% and reduction per cent ranged from 98.7 to 99.4% with an average of 99.2±0.4%. This is compared with untreated jar (control) where emerged adults of E. cautella percentage was 15.7% and mortality was 84.3%, where infestation and nutrition was observed outside and inside 30 dates fruits. Reduction% by of Ch. carnea larvae reached to 99.2±0.4%. Also there were nine Ch. carnea larvae reached to third nymph instar and only three of them reached the pupal stage. Statistical analysis showed that there were significant differences between treatments and the control.

These results indicated that Ch. carnea larvae had great influence on E. cautella eggs.

2 - Efficiency of T. evanescens against E. cautella eggs

After 30 to 45 days from artificial infested dates by E. cautella eggs, the percentage of emerged adult of *E. cautella* ranged from 0.1 to 0.4% with an average of 0.3±0.2% from eggs treated by *T. evanescens* with reduction percentage ranged from 97.8 to 99.5% with an average of 98.6±0.9%. In case of control the percentage of emerged *E. cautella* adults was 18.3% with 81.7% reduction. Infestation and nutrition was observed outside and inside dates fruits. Emergence percentage of *T. evanescens* ranged from 36 to 72% with an average of 50.7±18.9%. Table (2).

Statistical analysis showed that there were significant differences between treatment and control.

These results indicated that T. evanescens adults had great influence on E. cautella eggs.

These results are in accordance with those reported by Bakri (2008), Brower (1983 and 1984).

Bakri (2008) found that one day old egg of E. cautella was the most suitable age for parasitoid T. evanescens at different rates followed by the two days old and the least suitable one was the three days old.

3 -Efficiency of Bracon hebetor against Ephestia cautella larvae

Data presented in Table (3) revealed the effect of Bracon hebetor on E. cautella 3rd-5th larval instar.

After 30 to 45 days from artificial infested dates by E. cautella eggs, the percentage of emerged adult of E. cautella ranged from 0 to 6% with an average of 2±3.5%, parasitism percentage (reduction %) of B. hebetor ranged from 94 to 100% with an average of 98±3.5%. In case of control, percentage of emerged E. cautella adults was 100% and zero mortality. Emergence percentage of B. hebetor ranged from 36 to 87% with an average of 66.7±27.02%.

Statistical analysis showed that there were significant differences between treatment and control

These results indicated that B. hebetor had great influence on of E. cautella larvae, especially on 3rd, 4th and 5th larval instars.

These results agreed with Dawood (1967) who reported that a braconid parasite, Microbracon hebetor Say., was recorded as an internal and external parasite of E. cautella. Also, Keever et al. (1985) reported that Bracon hebetor Say. is a gregarious ectoparasitoid and an important biological control agent of several Lepidopteran stored product pests due to its rapid growth and development rates.

All the previous results indicated that use of Ch. carnea larvae and T. evanescens adults in controlling E. cautella eggs, as well as use of B. hebetor in controlling E. cautella larvae may be useful as a part of an integrated tamr pest management program based on biological agents.

Statistical analysis showed that there were no significant differences between the three treatments (Ch. carnea larvae, T. evanescens and Bracon hebetor) in controlling E. cautella eggs and larvae where F value was 0.237 and P value >0.05 (Table 4).

Means in each columns followed by different letters are significantly different from each other at $p < 0.05$.

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Tables

Table (1): Efficiency of *Ch. carnea* (Staph.) Larvae against *E. cautella* eggs.

Treatments	Number of eggs	Number of *Chrysoperla carnea* larvae	Emerged *E cautella* adult%	Reduction%	T value	P Value
Means	1000	10	o.1-0.2 0.13±0.06	98.7- 99.4 99.2±0.4	31.9	p<0.05
Control	1000	0.0	15.7	84.3		

Table (2) : Efficiency of *T. evanescens* against *E. cautella* eggs.

Treatment	No. of host eggs	No. of *Trichogramma evanescens*	Emerged *E. cautella* adult%	Reduction %	Emerged *Trichogramma evanescens%*	T value	P value
Mean±S.E.	1000	Card (1× 1 cm) (500 eggs)	0.1 – 0.4 0.3±0.2	97.8-99.5 98.6±0.9	36 – 72 50.7 ±18.9	16.9	P<0.05
Control	1000	0.0	18.3	81.7			

Table (3): Efficiency of *B. hebetor* against *Ephestia cautella* larvae

Treatments	No. of host larvae	No. of *Bracon hebetor*	Emerged *E. cautella* adult%	Reduction%	No. of emerged *Bracon hebetor*	T value	p value
Mean±S.E.	50	2 (Male+female)	0 – 6 2±3.5	94 – 100 98±3.5	36 – 87 66.7±27.02	24.5	P<0.05
Control	50		100	0.0			

Table (4): Statistical variance between the three treatments (*Ch. carnea* larvae, *T. evanescens* and *Bracon hebetor*) in controlling *E. cautella* eggs and larvae.

Treatments	The natural enemies			F. value	P. value
	Ch. Carnea	*T. evanescens*	*B. hebetor*	0.237	P> 0.05
Reduction%	99.2±0.4 a	98.6±0.9 a	98±3.5 a		

Biodiversity and seasonal abundance of mites associated with two varieties of date palm in Giza and Sohag Governorates, Egypt.

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ABSTRACT

The large number of mites are known associated with different varieties of date palm through out the world. Their distributional pattern is, however not constant everywhere, which varies according to climatic factors. These mite species could be either Phytophagous, Parasitism, Predatism, Phoresy in addition to Saprophagous and Fungivorous species. In this study 37 mite species representing 31 genera, under 17 families, resembling three Sub-orders, Actinedida; Gamasida and Acaridida. These mite species classified according to their feeding habits into four Categories; plant feeders which causing great damage for both leaves and fruits, parasitic and predaceous mites play an important role as biocontrol agents of different insect and mite pests and fungivorous as well as uncertain feeding behavior mites inhabiting date palm. The population fluctuation of Phytophagous mites; Eutetranychus orientalis; Oligonychus afrasiaticus (Tetranychidae); Raoiella indica; Phyllotetranychus aegypticus (Tenuipalpidae) on Sewi variety was higher than Zaghloul variety as well as in Giza than Sohag during the two seasons of 2010 and 2011. The population of predaceous and parasitic mites associated with different pests infesting date palm increase as well as the population of pests increase, therefore, the biocontrol agents suppressing the populations of different pests in both varieties of date palm in two localities

Key words: biodiversity of phytophagous, peredaceous and parasitic mites, date palm.

INTRODUCTION

Date palm (*phoenix dactylifera* L.) production is a world agricultural industry producing about 4.7 million tones of fruit in 1997. The date fruit, which is produced largely in the hot arid regions of southern Asia and North Africa is marketed all over the world as a remains on extremely important substance crop in most of disert regions. (FAO,1998). Date palm fruit produced in Egypt are considered the best date fruit varieties, which can be exported to foreign markets provided that the product qualities are most satisfactory, being free from infestation of pests and residues of pesticides, El –Dakroury et al 2002. The date palm and its fruits are subject to attacks by several insect and mite pests that are in most cases well adapted to the Oasis environment. Damage caused by pests is considerable and lead to economic losses. Biodiversity of mites associated with palm trees, phytophagous, predaceous, parasitic, fungivorous, phoretic and saprophagous mites are very important to through lights on.

The present work aims to study the biodiversity and seasonal abundance of the economic mite pests which cause a great damage to palm trees and associated predaceous, parasitic mites on Zaghloul and Sewi varieties in Giza and Sohag Governorates.

MATERIALS AND METHODS

An area of two feddans in both Giza an Sohag Governorates cultivated with Zaghloul and Sewi varieties, feddan for each variety, about ten years old, during the period of April to August 2010 and 2011 samples of 15 leaflets from three trees for each variety were collected. Fortnight intervals exchange between Giza and Sohag during the period of study from April to August2010 and 2011. Samples of 15 leaflets from three palms for each variety were collected randomly. Transferred to laboratory to examine using stereomicroscope.

The population fluctuation of different mite species were recorded during period of study for both varieties in two localities of Giza and Sohag Governorates speciemen of 2-3 individuals for each mite species were put in Nesbitt,s clearing agents, then mounted on glass slide using Hoyer,s medium for examination. Lables with necessary data were stuck on the slides .

RESULTS AND DISCUSSION

In this study 37 mite species belonging to 31 genera, representing 17 families under three suborders, Actinedida, Gamasida and Acaridida .Identification of mounted species were identify according to review given by Hughes, 1976., Zaher,1984, Zaher, 1986 and Mourya and Jamil 1982, Soliman et al (1973), and wafa et al (1986). These mite species were classified according to their feeding behavior into four group as follows:

Phytophagous mites

Suborder: Actinedida

1- FAMILY: Tetranychidae Donnadeiu. The date palm leaf brown mite *Eutetranychus orientalis* (Klein) which causes injury to leaf date palm trees. This mite species is feeding on upper leaf surface produces a multitude of gray spots, which gives leaves a chlorotic appearance. The infested leaves become weaken and finally drop. This mite species was recorded in rarely numbers in Giza and Sohag Governorates 2010 and the population increased during 2011 to moderate level, while, the date palm leaf brown mite E. orientalis population increased during the course study of 2011 in both localities Giza and Sohag on both Zaghoul and Sewi varieties Tabbles (1,5&6)

2-The mite date palm *Oligonychuin afrasiaticus* (McGregor) was collected in moderate numbers on both Zaghloul and Sewi varieties during the course study 2010 in Giza, while the population of mite, *O. afrasiaticus* was rarely on Zaghloul and moderate on Sewi varieties during the season 2010. On the other hand during the course study of 2011 sewi variety aggregated high numbers in Giza Table (1). Observation fieleds showed a heavy deposit of fine webbing collects dust. This mite species feed a long the midrib on lower surface of leaves causing yellowish patches at the points of attack. The mite, *O . afrasiaticus* feed on dates produces scar tissues on date skin, causing it harden crack and shrivel with subsequent reduction in fruit grade marketing. Population of mites increasing during July and August Table (5&6).

2-FAMILY : Tenuipalidae Berlese. The red palm,*Raoiella indica* Hrist . Also, known as the coconut mite. Data palms appear to be the most severely injured, *R. indica* lives on abaxia (lower)surfaces and is usually found on the under side of the leaves of the host plant in very large numbers. All active stages of the mite are dark red in colour with black markings. Attacked leaves display severe yellowing.

The population fluctuation of R. indica during this study showed that the both Zaghloul and Sewi varieties were moderate levele infestation in Giza during 2010 and 2011, while zaghloul variely during the period of study 2011 in sohag aggregated the high numbers comparing with 2010 season whereby it was moderate infestation Sewi variety was moderate infestation 2010 and high infestation 2011. Tabble (1,5&6). The tenuipalped mite, *Phyllotetranychus aegypticus* Sayed was recorded in the highest numbers on leaves of both varieties during the season 2010 and 2011 in the two localities except on zaghoul variety in Giza locality was in moderate level infestation.

The infestation oh *Ph. Aegypticus* symptoms appears difler from that of *R. indica* by whiches blotches due to the aggregation of mites for their which fanlike setage. The heavy mite infestation produce sufficient webbing. High temperature is favor mite development, therefore, population of this mite in sohag locality was highest than population in Giza locality Table (1,5&6).

3- FAMILY : Tarsonemidae Kramer. Some sprcies of tarsomemid mites had become serious pests on different crops. These mite species, *Polyphagotarsonemus latus* (Banks) and *stenotarsonemus spirifix* March were recorded on Zaghloul and Sewi varieties in Giza and Sohag Governorates. Tables (1). Pena et al 2006 reported that the red palm mite *R. indica* is an important pest of coconut, date palms and other palm species. Flechtmann and Jean Etienne 2004 reported that R. indica threat to palms in the Americas. El-Dakroury *et al.,* 2002 montioned that date palm liable to be infested with so many insect and mite pests. Zaher 1984 studied on phytophagous mitesin nile valley and Delta. Wafa et al (1968-1969) surveyed the occurrence of 18 mite speiec belonging to nine genera of ten uipalpid mites in U.A.R. and Giza

Predaceous mites

Suborder: Gamasida

1-FAMILY : Phytoseiidae Berlese. The Phytoseiid mites were represented by two predators associated with different pests infesting date palm trees. *Euseius Scutalis* A-H was recorded on both date palm varielies in few numbers in Giza and Sohag localities. *Amblyseius swirskii* (A-H) found in moderate numbers in Giza and high numbers in sohag on both varieties

2- FAMILY : Ascidae Voigts & Oudemans. Four predatory mite species of family acides were recorded associated with pests infested date palm trees in bothe Giza and Sohag localities in rarely numbers during the periods of study 2010 1nd 2011 years . Table(2).

3- FAMILY : Laelapidae Berlese. 1- *Androlaelaps casalis* Berlese, 2-*Hypoospis miles* Berlese, 3- *H. Sardo* Berlese. Three mite speieces belong to family laelapidae recorded between rarely and moderate numbers on Zaghloul and Sewi varialies in two localities Giza and Sohag Governorates.

4-FAMILY : SeJidae Berlese. The predatory mite, *SeJius paloghi*, the only one species of Sejid mites recorded during 2011 on two varieties of date palm trees in rarely numbers in Giza and Sohag Governorates.

5- FAMILY : Macrocgelidae vitzthum. 1- *Macrocheles Carintus* Koch, 2- *M. Mascaedomestica* Scopti, 3- *Gllyptholaspis confuse* (Fao). The macrochelid mites play an important role as a bio-control agent which suppressing the different pests poupulation on different crops, as well as date palm trees.

6-FAMILY: Uropodidae Berlese. Two Uropodid mite species, *uropoda minima* Kramer,was recorded in moderate numbers, while, *Chiropluropoda bakeri* Zaher &Afifi recorded in rarely numbers on both varieties and localities.

Suborder Actinedida

1- FAMILY: Cheyletidae leach. The Chelelid mites represented by three species which recorded on Zaghloul and Sewi varieties in both localities Giza and Sohag during the 2010 and 2011 years Table(2).

2- FAMILY: Cunaxidae Thor. *1- Cunaxa carpeolus* Berlese, 2- *Pulaeus Zaheri* El-Bishlawi&Rakha The predatory mites of Cunaxidae were recorded in rarely numbers Table (2).

3- FAMILY: stigmaidae oudmans . *1- Agistemus exsertus* Gonzlis . This predatory mite found in moderate numbers associated with pests infesting date palm varietes in two localities in Giza and Sohag governorates, 2. *aficanus* Soliman & Gomaa. The predatory mite species was recorded in rarely numbers on zaghloul and Sewi variettes in Giza and Sohag governorates . Table (2)

4- FAMILY: Tydeidae Kramer. Tow predatory mite speies were recorded, whereas, *Pronematus mhignitus* found in high numbers, while *Tydeus Californicus* found in moderate numbers associated with different pests infesting date palm trees under investigation in Giza and Sohag governorates . Table (2)

Suborder : Acaridida

1- FAMILY: Hemisarcoptidae, Oudemans. The predatory mite, *Hemisarcoptes malus* (Shimer) recorded in moderate numbers associated with scale insects in fasting date palm trees in Giza and Sohag governorates. The predatory mites associated with different pasts attracted many authers, El-Halawany et al 1986 recorded *H. malus* as a predatory mite on scale inserts, Attia et al 2012 studied on the predaceous mites associated with scale insects and other pests infesting mango trees at Qalubia Governorate, They found that the predatory mite *H.malus* considered one of the most biocontrol agents of diaspidid scale insects. Taha 1985 identified and described 15 mite species belong to suborders Astignta, prostigmata and mesostigmata . Sallam et at 2007 studied the predatory insects, mites and spiders associated with pests infesting date palm in Rashid region, El-Beheira Governorate . Soliman et al 1973 Survey 15 predaceous mites belonging to six families found associated with different pests infesting fruit trees Thomas and Timothy 1999 provide an overview of an integrated management program for pests installed palms.

Parasite mites

The parasitic mites play an important role in controlling some insect pests associated with date palm trees . Sally et al 2013 identified uropodid mite *Aegyptus rhynchophorus* as a parasitic on pupae and adults of the red palm weevil *Rhynchophorus ferrugineus* (Olivier), Al-Dhafar and Al-Qahtani 2012 recorded three mite species were found on date palm, one of them which *Aegyptus alhassa* new.sp as a parasitic collected from egg, Lurrue and pupae of the red palm weevil *R.Farrugineus.*

In this study the uropodid mite, *Leiodinychus Karmeri* was recorded associated with pupae and adults of the red palm weevil *R.Ferrugineus* and the two pyemotid mite species, *Pyemotes herfici and P.tritic* were recorded associated with some insects. Hassan et al 2011 recorded thirteen mite species associated with adults and pupae of *R.Ferrugineus* in Ismailia governorate .

Fungivorous mites

Three mite species of family (Acaridae.Acaridida) were recorded of leaves of date palm of the two varieties, Zaghloul and Sewi in both localities Giza and Sohag . These mite

species, *Tyrophegous Putrescentiae* which found in moderate numbers on two varieties and localities, *T.entomophagus* and *Mycetoglyphus funginorus* were found rarely numbers during 2010 and in moderate numbers during 2011.

Aboundance of four phytophagous mites infesting date palm varieties in Giza and Sohag Governorates:

1- The date palm leaf brown mite, *Eutetranychus orientalis*. The aboundance of mites (numbers of motle stages/inch) of *E.orientalis* on zaghloul and sewi varieties of date plam in Giza and Sohag governorates. The investigation period extended between April and Augest in the Two successive years 2010 and 2011. As shown in Table (5) The population aboundance of this mite species started in few numbers in April, then increased to its maximum in Augest on both Zaghloul and sewi varieties although the level infeslation of sewi was 1.4 times than zaghloul variety in Giza while in Sohag, it was 1.6 times during the season 2010. The population curve of *O.afriasiaticus*, *R.indica* and *phyleotetranychus aegypticus* on the two varieties showed almost the same trend during the two studied seasons. The population of these phylophagous mites, were 1.5, 1.4 and 1.2 times on Sewi variety than zaghloul variety in Giza location at the same pattern. In sohag region data revealed that during the season 2010 population fluctuation of different phytophegous mite species were high on Sewi than zaghloul variety by 1.6,1.1,1.2,1.1and 1.6 for *E.orientalis, O.afriaticus, R.indica* and *ph.aegyptious respectrely* . Table (5). As shown in (table 6) during the season 2011 obtained data revealed that the Sewi variety aggregated high numbers than zaghloul variety in both localities excyet the date plam lcaf brown mite *E.orientalis* the total numbers of mites on Sewi variety equal 0.7 of the population on zaghloul variety Table (6) zaher et al 1969 carried out biological studied on the red palm *R.indica* and *phyllotetranychus aegyptiacus* infesting date palm trees. Zaher, 1984 studied the ecology of phylephagous, predaceous and sail mites in nile valley and delta .

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Tables

Table (1): Incidence of Phytophagous mites infesting date plam trees in Giza and Sohag Governorates, Egypt, during 2010 &2011.

Suborders Families	Species	Abundance							
		Giza				Sohag			
		2010		2011		2010		2011	
		Z	S	Z	S	Z	S	Z	S
Atinedid Tetranychidae Donnadeiu	*Eutetranych orieutoilis* (klein)	+	++	++	++	+	++	++	++
	Oilgonychus afrsiaticus (MCGregor)	++	++	++	+++	++	+	++	++
Tenuipulpidae Berlese	*Raoiella indica* Hirst	++	++	++	++	++	++	+++	+++
	Phyllotetranychus aegyptiacus Sayed	++	+++	+++	+++	+++	+++	+++	+++
Tarsonemidae Kramer	*Polyphagotarsonemus latus* (Banks)	+	++	++	+++	+++	+++	++	+++
	Stenotarsonemus Spirifix March	+	++	++	+++	++	+++	++	+++

Z=Zaghloul variety S= Sewi variety + = Rare (1-2 individuals inch) ++=Moderate (3-4 individuals inch) +++= High (more than individuals)

Table (2): Incidence of predaceous mites collected from date plam trees in Giza and Sohag Governorates, Egypt, during 2010 &2011.

Suborders &Families	Species	Abundance			
		Giza		Sohag	
		2010	2011	2010	2011
Gamasida Phytoseiidae Berlese	Euseuis scutalis A-H	+	+	+	+
	Amblyseeius swirskii(A-H)	++	++	+++	+++
Ascidae Voigts &Oud.	Blattisocius keegani(Fox)	+	+	++	++
	Lasioseius bispnous Evans	+	+	++	++
	Proctolaelaps pygmaseus Muller	+	+	+	++
	Melichares ornate Berlese	+	+	+	+
Laelapidae Berlese	Androlaelaps casalis Berlese	+	++	+	++
	Hypoaspis mites Berlese	+	++	+	++
	Hypoaspis sardo Berlese	+	+	+	
Sejidae Berlese	Sejius paloghi	-	+	-	+
Macocholidae Vitzhum	Macrocheles carintus Koch	++	++	++	++
	Macrocheles mascaedomestica Scopli	+	++	++	+++

Suborders &Families	Species	Abundance			
		Giza		Sohag	
		2010	2011	2010	2011
	Glyptholaspis confusa (Fao)	+	+	+	+
Uropodidae Berlese	Uropoda minma Kramar	++	+++	++	+++
	Chiropluropoda bakeri Zaher&Afifi	+	+	+	+
Actinedidae Cheyletidae Leach	Cheyletus malaccensis Oud.	++	++	++	++
	Cheyletus fortis Oud.	+	+	++	++
	Cheyletogenes ornatus (Can&Fons.)	+	+	+	++
Cunaxidae Thor.	Cunaxa carpeolus Berlese	+	+	+	+
	Pulaeus zaheri (El-Bishlawi& Raleha)	+	+	+	+
Stigmadae Oud.	Agistemus axertus Gonz	++	++	++	++
	Agistemus africanus soliman &Gomaa	+	+	+	+
Tydeidae Kramer	Pronematus ubiquitus McGregor	+++	+++	+++	+++
	T ydeus califrnicus (Banks)	++	+++	++	+++
Acaridida Hemisarcoptidae	Hemisarcoptes malus (Shimer)	++	++	++	+++

+ = Rare (1-2 individuals Leaf) ++=Moderate (3-4 individuals Leaf) +++= High (more than individuals Leaf)

Table (3): Incidence of parasitic mites associated with pests infesting date plam trees in Giza and Sohag Governorates, Egypt, during 2010 &2011.

Suborders&Families	Species	Abundace			
		Giza		Sohag	
		2010	2011	2010	2011
Actinedidae Pyemotidae Oud.	Pyemotes herfici(Oud.)	+	++	+	++
	Pyemotes tritci(La-Gree-Fossat&Mantane)	+	++	+	+++
Gamasida Uropodidae	Leiodinychus karmoeri (G.&R.,Canestrini)	+	++	+	++

+ = Rare (1-2 individuals Leaf) ++=Moderate (3-4 individuals Leaf) +++= High (more than individuals Leaf)

Table (4): Incidence of fungivorus mites collected from leaflets of date palm trees in Giza and Sohag Governorates, Egypt, during 2010 &2011.

Suborders&Families	Species	Abundace			
		Giza		Sohag	
		2010	2011	2010	2011
Acanididae Acaridae Ewixg&Nesbitt.	Tyrophagus putrescentiae (Sharnk)	++	++	++	++
	Tyrophagus entomopgagus	+	++	+	++
	Mycetoglyphus fungivorus Oud.	+	++	+	++

+ = Rare (1-2 individuals Leaf) ++=Moderate (3-4 individuals Leaf) +++= High (more than individuals Leaf)

Table (5): Seasonal Abundance of phytophagous mites infesting two varieties of date palm in two governorates during 2010.

Inspection date		Zaghloul variety								Sewi variety							
		Giza				Sohag				Giza				Sohag			
		E. orientalis	*O. afrsiaticus*	*R. indica*	*P. aegypticus*	*E. orientalis*	*O. afrsiaticus*	*R. indica*	*P. aegypticus*	*E. orientalis*	*O. afrsiaticus*	*R. indica*	*P. aegypticus*	*E. orientalis*	*O. afrsiaticus*	*R. indica*	*P. aegypticus*
April	1	12	22	18	26	8	15	16	19	24	39	35	48	21	18	22	35
	15	16	26	22	28	10	18	21	21	33	45	38	56	25	21	31	42
May	1	19	39	32	43	13	21	30	28	38	48	42	75	32	22	35	48
	15	25	42	35	56	15	32	33	33	41	54	45	68	35	35	39	61
June	1	31	54	44	72	22	35	35	39	45	62	49	85	41	36	45	77
	15	37	62	51	78	25	41	45	45	35	75	58	94	40	48	44	68
July	1	42	71	54	85	28	44	49	48	52	78	76	101	42	49	56	74
	15	53	76	60	94	33	52	55	58	66	87	89	105	48	56	60	84
August	1	58	85	62	111	38	56	59	57	75	89	98	111	50	68	68	91
	15	62	104	68	129	48	68	62	55	89	94	115	125	66	75	75	96
Total		355	581	446	717	240	385	405	403	498	671	645	868	400	428	475	676
Mean		35.5 b	58.1 ab	44.6 b	71.7a	24 b	38.5 a	40.5 a	40.3 a	49.8 b	67.1 ab	64.5 b	86.8 a	40 b	42.8 b	47.5 b	67.6 a
LSD		**22.67**				**13.91**				**21.48**				**16.24**			

Table (6): Seasonal Abundance of phytophagous mites infesting two varieties of date palm in two governorates during 2011.

Inspection date		Zaghloul variety								Sewi variety							
		Giza				Sohag				Giza				Sohag			
		E. orientalis	O. afrsiaticus	R. indica	P. aegypticus	E. orientalis	O. afrsiaticus	R. indica	P. aegypticus	E. orientalis	O. afrsiaticus	R. indica	P. aegypticus	E. orientalis	O. afrsiaticus	R. indica	P. aegypticus
April	1	19	32	23	55	5	20	32	72	15	28	28	62	18	25	45	168
	15	29	45	35	59	11	18	39	69	22	44	30	66	21	32	52	188
May	1	31	48	38	67	13	28	44	85	19	59	40	75	33	38	66	201
	15	38	53	42	68	18	31	49	88	33	62	45	89	45	41	78	218
June	1	42	64	55	81	25	38	58	95	41	77	61	32	48	45	89	249
	15	45	69	64	84	38	49	84	111	48	89	68	99	49	49	94	277
July	1	54	72	63	114	44	50	94	121	45	94	69	112	50	48	97	312
	15	61	72	68	95	59	63	105	131	49	98	75	10	52	58	104	345
August	1	68	78	75	108	62	75	111	142	54	101	88	122	68	65	112	380
	15	75	88	88	145	65	98	122	155	58	111	96	125	75	78	122	415
Total		462	627	551	876	340	470	738	1069	339	763	600	947	459	479	859	1140
Mean		46.2 b	62.7 b	55.1 b	87.6 a	34 c	47 c	73.8 b	106.9 a	38.4 b	76.3 a	60 ab	94.7 a	45.9b	47.9 b	85.9 b	275.3 a
LSD		19.41				25.49				42.72				41.76			

The same letters at the same Governorat are not siginivicanlaty different

Figures

Eutetranychus orirntalis

Eutetranychus orirntalis

Oligonrchus afrasaitius

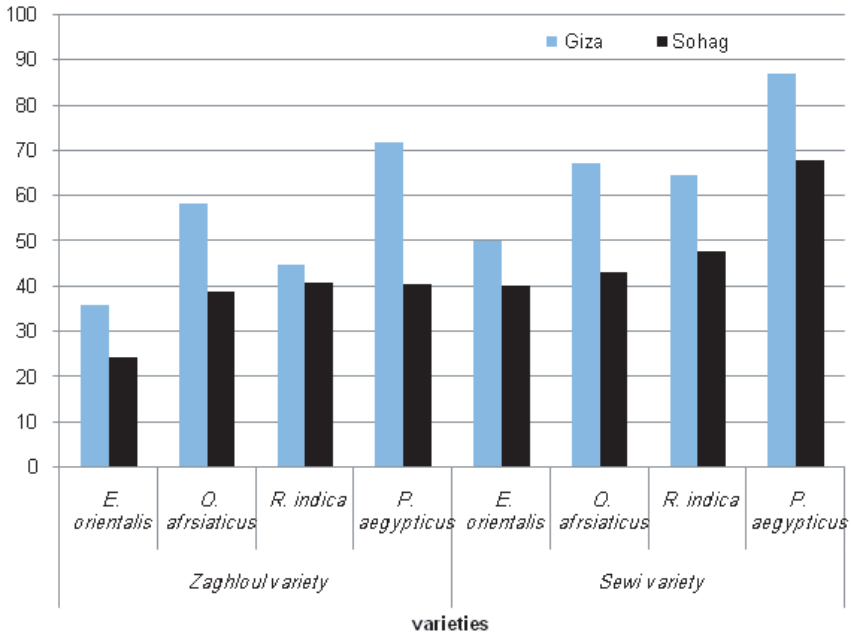

Fig. (1) Average mean numbers of phytophagous mites infesting two varieties of date palm in two governorates during 2010

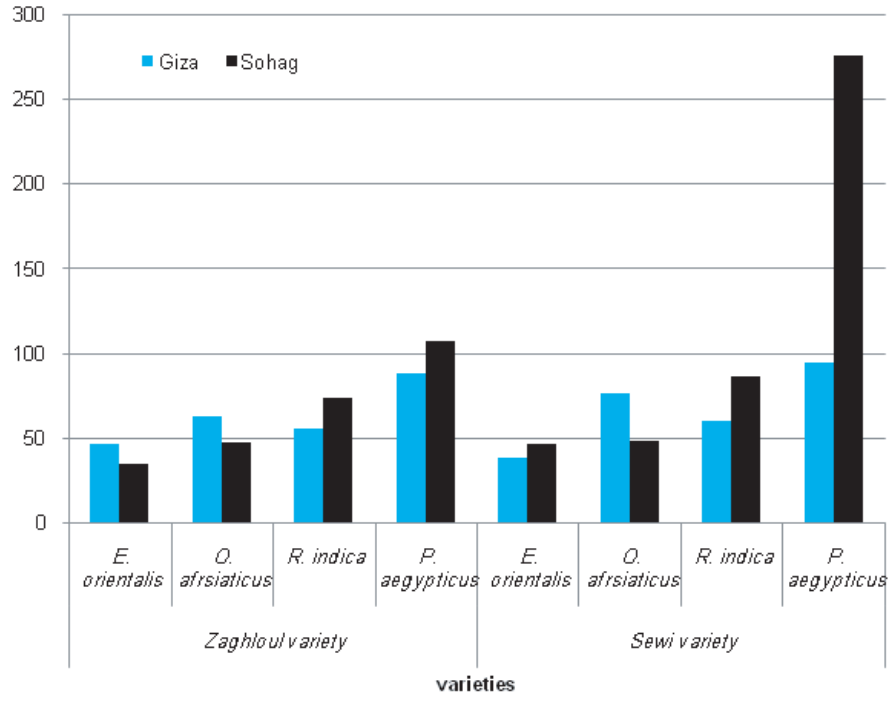

Fig. (2) Average mean numbers of phytophagous mites infesting two varieties of date palm in two governorates during 2011

Contribution to the study of effect of (Glycyrrhiza glabra L.) in region M'LILI (South of Algeria) on some chemical parameters of two varieties of date palms (*Phoenix dactylifera L.*)

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ABSTRACT

The objective of our work was to determine the nutritional status of date palms with the presence of licorice.

For this we have tried to assess the impact of licorice (Glycyrrhiza glabra L.) on the mineral nutrition of two varieties of date palms(*Phoenix dactylifera* L.) in the region M'lili (W. Biskra south of Algeria) by the method of foliar diagnosis completed and the determination of total sugars.

Mineral nutrition of date palm requires availability in contact with roots, sufficient amounts of nutrients can be absorbed at a rate corresponding to the current needs of the plant. However, many processes in the soil and the effect of climate and the presence of competing plants can alter the availability of these nutrients and their levels in the plant.

The conclusions that we can make with regard to the results obtained are as follows :

A low total sugars in fruits and leaves in varieties of dates with the presence of licorice.

A light foliar results and their interpretations, we can conclude that nitrogen nutrition is high for both varieties of dates especially with the presence of licorice (Rhizobium).

High foliar concentrations of Calcium, magnesium and sodium for both varieties of dates, especially with the presence of licorice.

Low foliar iron content in varieties of dates with the presence of licorice.
Low foliar concentrations of potassium, phosphorus, boron, copper and zinc for both varieties of dates with and without licorice .

Key words: *Phoenix dactylifera* L., *Glycyrrhiza glabra* L, Mineral nutrition, total sugars, varieties

INTRODUCTION

The date palm is both the symbol and the backbone of the oasis ecosystem. It creates a microclimate promote the development of underlying cultures. The date palm is for the people of the Sahara that the olive tree For the Mediterranean: a source of a providential fruit. Algerian palm hosts a rich and diverse germplasm with more than 13 million palm trees and 940 cultivars identified [1].

Dates are subject to significant business activity in particular the famous Deglet Nour which occupies 52.87% of the national production. In Algeria production exceeded 7 million quintals for the 2010 agricultural companion [2], 65% of the national production was done by two wilayas, Biskra with 35% and El Oued with 30% [3].

Licorice (*Glycyrrhiza glabra* L.) is a perennial plant of the family Fabaceae under: family Fabaceae, aromatic roots. It is native to southern Europe and Asia.

Only the roots and stolons are used in industry (pharmaceutical and food) because they are the richest bodies in active principles. Licorice can be used either in kind or in the form of crude extract. It is also used in confectionery and dermopharmacy [4].

And as licorice exists and dispersed in the Algerian oasis and plots dates, the aim of this study examines the effects of licorice (*Glycyrrhiza glabra*) on the growth and quality of production of the two varieties of dates (*Phoenix dactylifera*) "Deglet Nour" and "Mech-Degla" area M'lili, through dosages of total sugars in the pulp of dates, leaves and also the determination of minerals in the leaves of the two varieties of a part, and the comparison of these results with the results representing the date palms witnesses (without licorice) on the same region of another part.

MATERIALS AND METHODS

Were selected in each area three palm of each variety, and was ready to consider the homogeneity of age, length, and vegetative growth through assays of total sugars in the pulp of dates, leaves and also the determination of mineral elements in the leaves of both varieties on the one hand, and comparing these results with the results of palm trees that represent the controls (without licorice) on the same area on the other hand.

1.Introduction of the study area:

M'lili: Extends over an area of 371.80 km2.

Limits:

-North: the town of El Hadjeb

-North-West: the common Bouchagroune

-South: (Steel) wilaya of El Oued.

-to the West: common Ourlal

-to the east: the common Oumache.

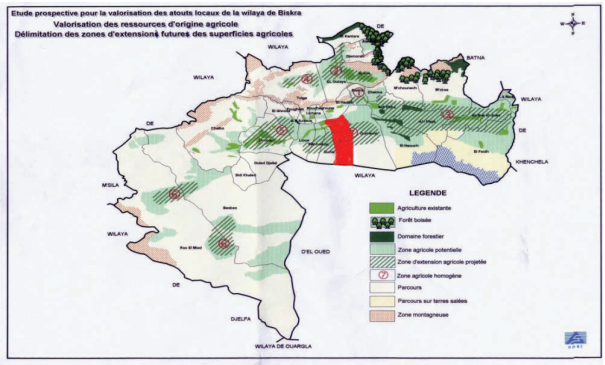

Figure 1: Location of the Common M'lili in the wilaya Of Biskra (ANAT)

2. Dosage the total sugars

The method of Dubois et al (1956) used to assay the oses using phenol and concentrated sulfuric acid and a solution mother glucose as standard, in the presence of these two reactants, oses give a yellow-orange color, the intensity is proportional to the carbohydrate concentration, the optical density was determined between 450 and 550 nm [5].

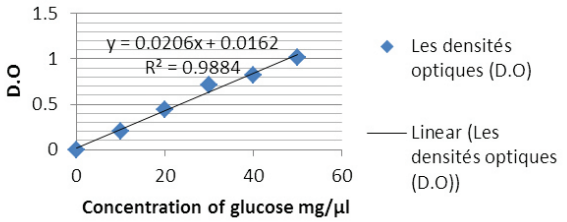

Figure 2: Calibration Curve of dosage the total sugars

The concentrations of total sugars in dates and leaves are determined from calibration curve.

3. Dosages of mineral elements in the leaves

Prior to mineral analyzes of plant samples were dried at 60 ° C to constant weight and then crushed to the average of a planetary mill to obtain a fine grind.

3.1Principe

Mineralization by acid attack leaves powders with heating by microwave, closed system.

3.2. Mode operative:

1. Weigh about exactly 0.001g near 0.4 g of powdered leaf comminuted to 500 microns.
2. Record the exact weight on the worksheet
3. The plant previously dried must be crushed with a grinder capable of reducing the sample in its entirety to a fineness of less than 500 µm.
1. Transfer to a Teflon tube numbered.

2. Add 5 ml of HNO3 at 56% by using a dispenser
3. Add 5 ml of 30% H2O2 with a distributor
4. Close tubes hermetically with a screw cap.
5. Book a without sample tube which is engaged the thermal probe apparatus for controlling the heating temperature.
6. Place the tubes on the plate supporting the samples (never put less than 10 tubes) and place the tray in the oven. Close and secure.
7. Start the microwave oven and the heating cycle.

Table 01: Steps of the mineralization cycle I

Step	Time in min	Power Watts	Temperature ° C
1	10	1000	110
2	15	1200	160
3	10	1200	180
4	25	0	50

At the end of the cycle of mineralization:

1. Once the cooled tubes.
2. Remove the tray from the oven.
3. Unclogging tubes for each sample, using a wash bottle of demineralized water.
4. Get the contents of the tube through a filter without cinder previously washed and dried.
5. The filter is placed on a very clean glass funnel with a long shaft.
6. Collect the filtrate in a 50 ml volumetric flask.
7. Wash well inside the tube with jets of spray, then rinse 2 times the filter.
8. Gauge to 50 ml with demineralized water.
9. If there remains no deposit at the bottom of the tube, making the mineralisate pass directly into the flask by rinsing well inside the tube with jets wash bottle and gauge 50 ml with demineralized water.
10. Stopper the flasks.

3.3. Oven programming:

Decontaminate tubes mineralization practicing mineralization cycle as follows and added with 10 ml in each tube only (2 x 5 ml) of 56% nitric acid.

Table 02: cycle stages of mineralization II

Step	Time in min	Power Watts	Temperature ° C
1	10	1000	110
2	15	1200	160
4	25	0	50

Rinse each tube with deionized water. Let drain the tubes.

3.4. Dosage

Be assayed elements: P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn and B in plasma emission spectrometry.

3.5. Analysis of nitrogen in plants

Take a test sample approximately 0.5 g, well hidden in a piece of aluminum foil and making the injection into the furnace of elementary analyzer LECO nitrogen.

The LECO analyzer is a device that gives the content of nitrogen contained in the sample injected into the furnace by infrared rays, the detection is by a thermal conductivity detector.

The apparatus is supported by calculation software and the result is displayed directly on the screen of the PC% nitrogen (agronomic laboratory Fertial, 2013).

4. Statistical analysis:

The results obtained are expressed as the mean plus or minus standard deviation (m ± s). Statistical analysis was performed using the Mini tab (WEISBERG, 1985) software, and present the results in the form of histograms and curves (EXEL).

RESULTS AND DISCUSSION

1. Results

1.1. Determination of total sugars

1.1.1 Assay of total sugars in dates

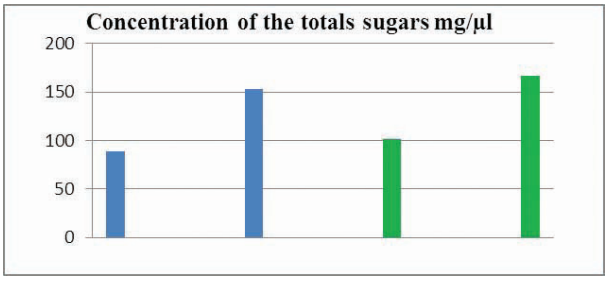

Figure 3: Histogram concentrations of total sugars in dates

The results obtained under the conditions of this experiment showed significant effects of licorice on total sugars in the dates of the two varieties of date palm.

According the ANOVA with two factors (Annex 1) we has classified date palms according to the means into two groups which are:

- Date palms without licorice are represented by the highest average (160.115 mg / µl).
- Date palms with licorice are represented by the average low monk (95.290 mg / µl).
- Among our results shown in the Annex 1, the varieties were classified according to the means of two groups are:
- The Mech-Degla variety is represented by the highest average (133.92 mg / µl).
- Deglet Nour variety is represented by the average low monk than Mech-Degla variety (121.485 mg / µl).

1.1.2. Dosages of sugars in the leaves

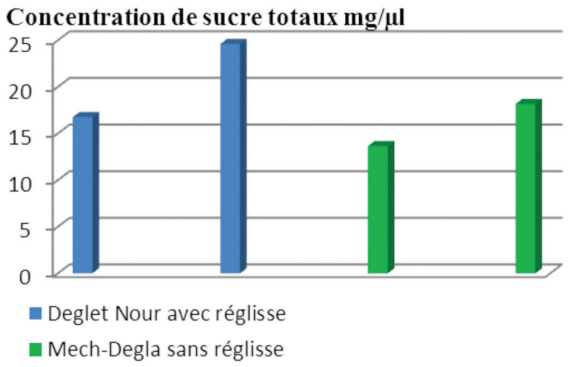

Figure 4: Histogram the concentrations of total sugars in the leaves

The results obtained under the conditions of this experiment showed significant effects of licorice on total sugars in the leaves of two varieties of date palm.

According the ANOVA with two factors (Annex 2) were classified date palms according to the means in two groups are:

- Date palms without licorice are represented by the highest average (21.39 mg µl).
- Date palms with licorice are represented by the average the low monk (15.20 mg / µl).

Among our results shown in the Annex 2, the varieties were classified according to the means of two groups are:

- Deglet Nour variety is represented by the highest average (20.67 mg / µl).
- The Mech-Degla variety is represented by the average the low monk than Deglet Nour (15.91 mg / µl).

1.2 Dosages of mineral elements in the leaves

1.2.1 N (nitrogen) in% (1% = 10g/kg)

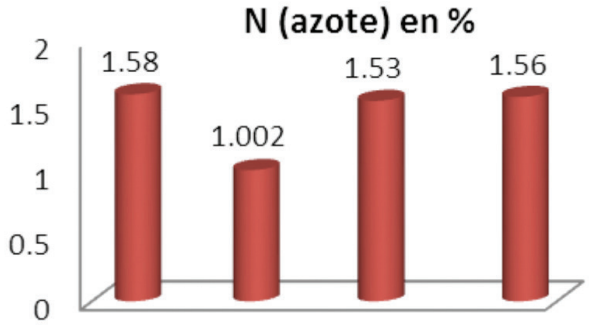

Figure 5: Concentrations of nitrogen in two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on the nitrogen in the leaves of two varieties of date palm.

According ANOVA with two factors (Annex 3) were classified date palms according to the means in two groups are:

Date palms with licorice are represented by the highest average (1.555%).

- Date palms without licorice are represented by the average the low monk (1.28%).

Among our results shown in the Annex 3, the varieties were classified according to the means of two groups are:

- The Mech-Degla variety is represented by the highest average (1.545%).
- Deglet Nour variety is represented by the average the low monk than Mech-Degla variety (1.291%).

1.2.2 Ca (calcium) in % (1%= 10g/kg)

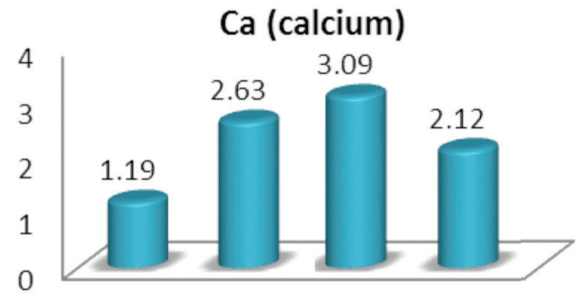

Figure 6: Calcium concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on the Calcium in the leaves of two varieties of date palm.

According to the ANOVA with two factors (Annex 4) were classified date palms according to the means in two groups are:

Date palms without licorice are represented by the highest average (2.38%).

- Date palms with licorice are represented by the average the low monk (2.14%).

Among our results shown in the Annex 4, the varieties were classified according to the means in two groups are:

- ü The Mech-Degla variety is represented by the highest average (2.61%).
- ü Deglet Nour variety is represented by the average the low monk than Mech-Degla variety (1.91%).

1.2.3 Mg (magnesium) in % (1%= 10g/kg)

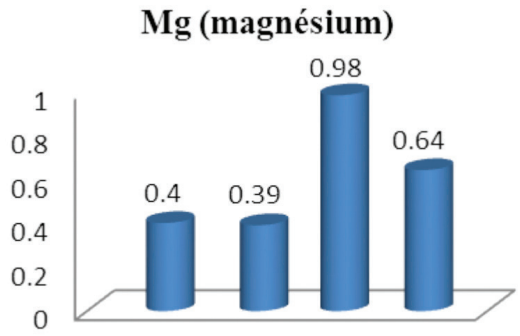

Figure 7: Magnesium concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on Magnesium in the leaves of two varieties of date palm.

According to the ANOVA with two factors (Annex 5) were classified date palms according to the means in two groups which are:

- Date palms with licorice are represented by the highest average (0.690%).
- Date palms without licorice are represented by the average the low monk (0.515%).

Among our results shown in the Annex No. 5, were classified according to the means varieties into two groups which are:

- The Mech-Degla variety is represented by the highest average (0.810%).
- Deglet Nour variety is represented by the average the low monk than Mech-Degla variety (0.395%).

1.2.4. P (phosphor) in % (1%= 10g/kg)

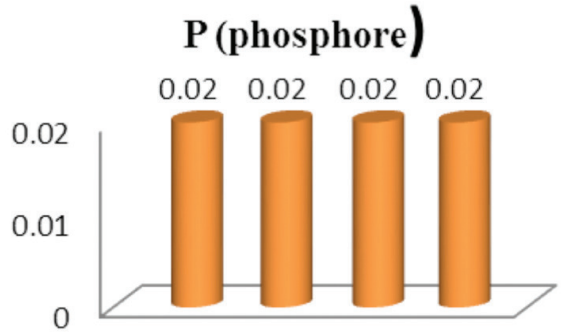

Figure 8: Phosphor concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed no effect of licorice on the Phosphor in the leaves of two varieties of date palm.

According to the ANOVA with two factors (Annex 6) and Figure No. 28, it was found that there's not a significant effect of licorice whatever the variety studied.

1.2.5 K (potassium) in% (1% = 10g/kg)

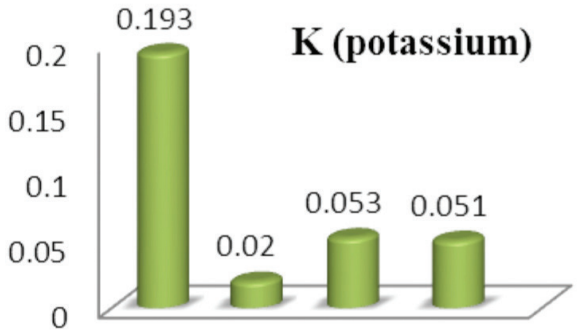

Figure 9: Potassium concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on Potassium in the leaves of two varieties of date palm.

According to the ANOVA with two factors (Annex 7) were classified date palms according to the means in two groups which are:

- Date palms with licorice are represented by the highest average (0.123%).
- Date palms without licorice are represented by the average the low monk (0.035%).

Among our results shown in the Annex No. 7, a class e varieties according to the means in two groups which are:

- Deglet Nour variety is represented by the highest average (0.106%).
- The Mech-Degla variety is represented by the average the low monk than Deglet Nour (0.052%).

1.2.6. Na (Sodium) in ppm (1ppm = 1mg/kg)

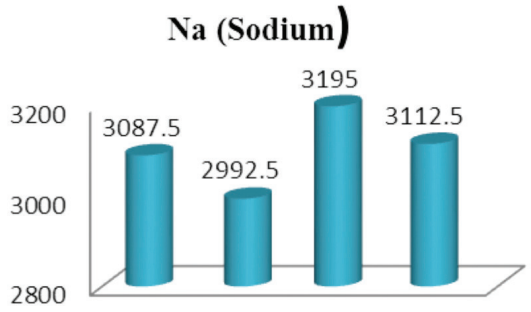

Figure 10: Sodium concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on the sodium in the leaves of two varieties of date palm.

According ANOVA with two factors (Annex 8) was ranked date palms according to means in two groups which are:

- Date palms with licorice are represented by the highest average (3141.3 mg / kg).
- Date palms without licorice are represented by the average the low monk (3052.5 mg/ kg).

Among our results shown in Annex No. 8 varieties were classified according to the means in two groups are:

- The Mech-Degla variety is represented by the highest average (3153.8 mg / kg).
- Deglet Nour variety is represented by the average the low monk than the Mech-Degla variety (3040.0 mg / kg).

1.2.7. Fe (Fer) in ppm (1ppm = 1mg/kg)

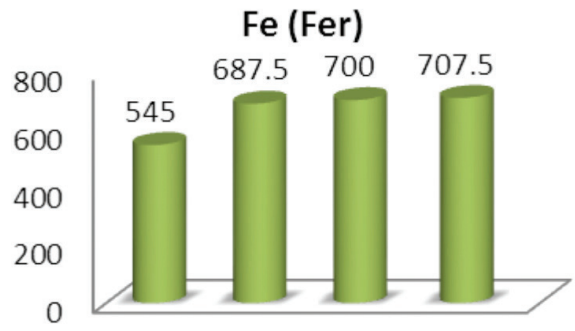

Figure 11: Fer concentrations in the two varieties of date palm

The results obtained under the conditions of this experiment showed significant effects of licorice on the Fer in the leaves of two varieties of date palm.

According to the ANOVA with two factors (Annex 9), were classified according to the average date palms into two groups which are:

- Date palms without licorice are represented by the highest average (698mg/kg).
- Date palms with licorice are represented by the average the low monk (623mg/kg).

Among our results shown in Annex No. 9 varieties were classified according to the means in two groups are:

- The Mech-Degla variety is represented by the highest average (704 mg / kg).
- Deglet Nour variety is represented by the average the low monk than Mech-Degla variety (616 mg / kg).

2. DISCUSSIONS

The levels of total sugars in the leaves and dates for both varieties Deglet Nour and Mech-Degla are low with the presence of licorice by contribution to the witness because the compound is of licorice glycyrrhizin with flavor 50-60 times as sweet as sugar crystallized and hydroxyglycyrrhizine (about 100 times as sweet as cane sugar), and sugars such as glucose (up to 4%), fructose, maltose, sucrose (2,4:6,5%) and polysaccharides (about 10%) [6].

The foliar nitrogen levels high in varieties of dates with licorice (norm composition of plant Annex 15) can be explained by the presence of rhizobia which are soil bacteria capable of inducing the roots of legumes (beans, peas, lens, peanut, soy, licorice, alfalfa, clover, lupine, glycine, rosewood ...) Only cyanobacteria and symbiotic Rhizobium bacteria [7] can use the nitrous air. Physiological studies have shown that there are three active transport systems for nitrate ions among these:

A system with high affinity constitutive (CHATS for "constitutive High Affinity Transport System") which absorbs nitrate when it is present in low concentrations in the rhizosphere (between 1µM and 1Mm) is the case of our study area (low soil nitrogen and organic matter).

The foliar sodium levels are high for both varieties with or without licorice, can be explained by: most has absorbed ions will be cross the wall and the cytoplasmic membrane but sodium is retained in significant amounts at these barriers. Other ions such as K^{+}, Cl^{-}, NO^{3-}or-PO_4O^{2-} migrate inwards [8].

Liquorice plant is a basophil and such as plants basophils consume a large amount of nutrients such as calcium and

magnesium which are strongly absorbed at higher pH values and higher than 7 [9], and in addition a saponoside glycyrrhizin is present as a mixture of salts: salts of calcium, magnesium and potassium, with a content of 3 to 5% of the mass of the dry drug [6], that clearly explains contents foliar calcium and magnesium are low in the Deglet Nour with liquorice and normal for the Mech-Degla variety. In Algeria, calcium deficiency has not been reported because soils generally contain sufficient quantities to meet the needs of plants [10].

The decreased cell permeability calcium and brakes thus the penetration of the water and most of the ions (K +, Fe) [8], what explains the low content of iron in varieties of dates with one hand liquorice and selective absorption de Fer by liquorice (intense chlorophyll activity in relation to dates). The results of leaf phosphorus contents and copper for both varieties with and without low and licorice are identical. That is to say there is no impact of the licorice plant phosphate nutrition on date palm but we can judge this case by:

Low leaf phosphorus levels also confirm the low levels of soil depth (in level the active roots) despite its high surface. [11] notes that this element is known for its very low depth migration and remains localized to the surface where it was brought. Alkaline soil pH (7.5 to 8.5) is frequently correlated bioavailability of difficulty by the plants of certain elements which there are essential such as phosphorus [12].

Low foliar potassium levels for both varieties of dates with and without licorice is explained by potassium fertilization of date palms in most of our arid regions has not answered. [13]

CONCLUSION

The results that we have put in evidence all the interest of foliar diagnosis and analysis of total sugars as tools of control of the nutrition of date palms. However, its reliability would increase with his work for several consecutive years.

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ANNEXES

Annex: 01

Two-factor ANOVA tested: HP depending Variety; Liquorice

Analysis of variance for TSD

Source	DL	SC	CM	F	P
Variety	1	463,89	463,89	8080,49	0,000
Liquorice	1	12606,84	12606,84	2,2E+05	0,000
Erreur	9	0,52	0,06		
Total	11	13071,25			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+----------				
DEGLA	121,485	(*)				
MECHD	133,920					(*
		----------+----------+----------+----------+----------				
		122,500	126,000	129,500		

Confidence interval 95%

RIG	Moyenne	-+----------+----------+----------+----------+----------+				
AVEC R	95,290	(*				
SANS R	160,115					*
		-+----------+----------+----------+----------+----------+				
		96,000	112,000	128,000	144,000	160,000

Annex: 02

Two-factor ANOVA tested: TSF depending Variety; Liquorice

Analysis of variance for TSD

Source	DL	SC	CM	F	P
VAR	1	68,116	68,116	73,71	0,000
RIG	1	114,763	114,763	124,19	0,000
Erreur	9	8,317	0,924		
Total	11	191,195			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+----------			
DEGLA	20,67			(----*-----)	
MECHD	15,91	(----*-----)			

		----------+----------+----------+----------+----------			
		16,00	17,60	19,20	20,80

Confidence interval 95%

RIG	Moyenne				
AVEC R	15,20	(---*---)			
SANS R	21,39				(----*---)
		----------+----------+----------+----------+----------			
		16,00	18,00	20,00	22,00

Annexe: 03

Two-factor ANOVA tested: N depending Variety; Liquorice

Analysis of variance for N

Source	DL	SC	CM	F	P
VAR	1	0,1935	0,1935	6,28	0,033
RIG	1	0,2252	0,2252	7,31	0,024
Erreur	9	0,2772	0,0308		
Total	11	0,6960			

Confidence interval 95%

VAR	Moyenne				
DEGLA	1,291	(----------*----------)			
MECHD	1,545		(----------*----------)		
		----------+----------+----------+----------+----------			
		1,200	1,350	1,500	1,650

Confidence interval 95%

RIG	Moyenne				
AVEC R	1,555			(----------*----------)	
SANS R	1,281	(----------*----------)			
		----------+----------+----------+----------+----------			
		1,200	1,350	1,500	1,650

Annexe: 04

Two-factor ANOVA tested: CA depending Variety; Liquorice

Analysis of variance for CA

Source	DL	SC	CM	F	P
VAR	1	1,449	1,449	2,99	0,118
RIG	1	0,166	0,166	0,34	0,573
Erreur	9	4,356	0,484		
Total	11	5,971			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+----------			
DEGLA	1,91	(----------*----------)			
MECHD	2,61		(----------*----------)		
		----------+----------+----------+----------+----------			
		1,50	2,00	2,50	3,00

Confidence interval 95%

RIG	Moyenne	---+---------+---------+---------+--------			
AVEC R	2,14	(----------*----------)			
SANS R	2,38			(----------*----------)	
		----------+----------+----------+----------+----------			
		1,60	2,00	2,40	2,80

Annexe: 05

Two-factor ANOVA tested: MG depending Variety; Liquorice

Analysis of variance for MG

Source	DL	SC	CM	F	P
VAR	1	0,51668	0,51668	56,93	0,000
RIG	1	0,09188	0,09188	10,12	0,011
Erreur	9	0,08167	0,00907		
Total	11	0,69023			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+-			
DEGLA	0,395	(--------*--------)			
MECHD	0,810				(--------*-------)

		----------+----------+----------+----------+-			
		0,450	0,600	0,750	0,900

Confidence interval 95%

RIG	Moyenne			
AVEC R	0,690		(----------*----------)	
SANS R	0,515	(----------*----------)		
		----------+----------+----------+----------+------		
	0,500	0,600	0,700	0,800

Annexe: 06

Two-factor ANOVA tested: P depending Variety; Liquorice

Analysis of variance for P

Source	DL	SC	CM	F	P
VAR	1	0,0000000	0,0000000	*	*
RIG	1	0,0000000	0,0000000	*	*
Erreur	9	0,0000000	0,0000000		
Total	11	0,0000000			

Confidence interval 95%

VAR	Moyenne				
DEGLA	0,0200000	*			
MECHD	0,0200000	*			
		----------+----------+----------+----------+-			
		2,00E-02	2,00E-02	2,00E-02	2,00E-02

Confidence interval 95%

RIG	Moyenne				
AVEC R	0,0200000	*			
SANS R	0,0200000	*			
		----------+----------+----------+----------+-			
		2,00E-02	2,00E-02	2,00E-02	2,00E-02

Annexe: 07

Two-factor ANOVA tested: K depending Variety; Liquorice

Analysis of variance for K

Source	DL	SC	CM	F	P
VAR	1	0,00891	0,00891	3,66	0,088
RIG	1	0,02297	0,02297	9,43	0,013
Erreur	9	0,02193	0,00244		
Total	11	0,05381			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+--			
DEGLA	0,106			(----------*----------)	
MECHD	0,052	(----------*----------)			
		----------+----------+----------+----------+--			
		0,035	0,070	0,105	0,140

Confidence interval 95%

RIG	Moyenne	------+----------+----------+----------+----------			
AVEC R	0,123			(----------*----------)	
SANS R	0,035	(----------*----------)			
		------+----------+----------+----------+----------			
		0,000	0,050	0,100	0,150

Annexe: 08

Two-factor ANOVA tested: NA depending Variety; Liquorice

Analysis of variance for NA

Source	DL	SC	CM	F	P
VAR	1	38817,2	38817,2	2981,16	0,000
RIG	1	23629,7	23629,7	1814,76	0,000
Erreur	9	117,2	13,0		
Total	11	62564,1			

Confidence interval 95%

VAR	Moyenne	----------+----------+----------+----------+-----			
DEGLA	3040,0	(*)			
MECHD	3153,8				(*)

		----------+----------+----------+----------+-----			
		3060,0	3090,0	3120,0	3150,0

Confidence interval 95%

RIG	Moyenne	--+----------+----------+----------+----------+				
AVEC R	3141,3					(-*)
SANS R	3052,5	(*)				
		--+----------+----------+----------+----------+				
		3050,0	3075,0	3100,0	3125,0	3150,0

Annexe: 09

Two-factor ANOVA tested: FE depending Variety; Liquorice

Analysis of variance for FE

Source	DL	SC	CM	F	P
VAR	1	22969	22969	15,12	0,004
RIG	1	16875	16875	11,11	0,009
Erreur	9	13669	1519		
Total	11	53513			

Confidence interval 95%

VAR	Moyenne	---------+----------+----------+----------+---------			
DEGLA	616	(----------*----------)			
MECHD	704			(----------*----------)	
		---------+----------+----------+----------+---------			
		600	640	680	720

Confidence interval 95%

RIG	Moyenne	-----+----------+----------+----------+----------			
AVEC R	623	(----------*----------)			
SANS R	698			(----------*----------)	
		-----+----------+----------+----------+----------			
		595	630	665	700

Use of ozonated water for controlling microbial contamination of date palm fruits

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ABSTRACT

Effects of treating date palm fruits with ozonated water (5, 15 and 30 minutes) on the general microbial contamination and on the added contamination with Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Aspegillus fumigates was investigated. Soaking date fruits for 15 minutes was effective to reduce general contamination with mesophillic aerobic bacteria, coliforms,molds and yeasts to levels that meet the requirements of Saudi Standards. The treatment was also found as the most effective for the reduction of added contamination with S. aureus, P. aeruginosa, E. coli and A. fumigates.

Key words: *Date fruits, preservation, microbial contamination, ozonated water.*

INTRODUCTION

Dates, like any other agricultural products, are subject to microbial contamination in the field and during handling processes. Studies conducted so far showed that date fruits in the rutab and Tamr stages are contaminated with many types of microorganisms (Abu-Zinada and Ali, 1982,Aidooetal.,1996,El-Sherbeenyetal.,1985, Nussinovitchetal.,1989, Hamad, 2008, Atia *et al.,* 2009 and Atia *et al.,* 2011). Potential spoilage microorganisms that contaminate date fruits include molds, yeasts and lactic acid bacteria, whereas potential pathogens include bacteria such as *Staphylococcus aureus* and yeasts such as *Candida pelliculosa*. Microbial contamination can lead to considerable losses in the crop especially at the *rutab* stage while contamination of the fruit at the tamr stage limits export chances, especially to the markets of the industrial countries. Treatments designed to control microbial contamination in date fruit processing factories in Saudi Arabia depend on washing with pure or chlorinated water. A survey conducted by the authors in a factory for date fruit processing that depend on washing with pure and chlorinated water showed that this treatment was not quite effective. In some cases the processed dates had higher microbial loads than the raw fruits (unpublished data).

The aim of this study was to investigate the effect of treatment with ozonated water on the microbial contamination of date fruits. Collected samples were treated with ozonated water by soaking for 5, 15 and 30 minutes. The effect of these treatments on the microbial load of the fruits was determined.

MATERIAL AND METHODS

Samples Collection

Date fruit samples of Khalas variety were purchased from one shop in Hofuf City, Saudi Arabia. Samples were collected in sterile containers and transferred to the laboratory for analysis and treatment.

Ozone Disinfection System

The Ozonated Water System FS-7200 (Biotek Ozone's Light Industrial Series FP-7200 Biotek Environmental Science Ltd.) was used. The system provides output flow rates of 600, 300 and 150 L/h, with ozone concentrations in the output of 1, 2 and 4 ppm, respectively. The 2 ppm ozone concentration was used in this study as recommended by the manufacturer.

Sample treatment

Treatment with ozonated water was performed by soaking 10 g date fruits sample in the ozonated water for 5, 15 and 30 minutes and then determining the microbial load. In a similar way 10 g date samples were soaked in sterile tap water to determine the effect of washing.

Microbiological analysis

Date fruit samples (10g) were weighed into sterile stomacher bags, 90 ml sterile peptone water (Oxoid, CM0009) added, homogenized in a stomacher (Lab-Blender 400, Seward Medical, England) for 45 seconds and aliquots (1.0 or 0.1 ml) plated out in duplicate as 10-fold dilutions in peptone water. Aerobic mesophillic bacteria were counted on plate count agar medium (PCA Oxoid, CM0325) incubated at 30˚C for 2 to 3 days, coliforms on violet red bile agar medium (VRBA Oxoid, CM0107) at 37˚C for 24 hours, yeasts and molds on PDA plates at 20 -30˚C for 3 to 7 days. *Escherichia coli* was cultivated on nutrient agar (CM0003, Oxoid), *Staphylococcus aureus* on baird-parker agar base (CM0961,Oxoid), *Pseudomonas aeruginosa* on pseudomonas agar base (CM0559) and *Aspergillus fumigatus* on potato dextrose agar (CM0139, Oxoid).

Contamination of fruits with selected microorganisms was performed as follows: the microorganisms were grown 2 days in Petri dishes containing appropriate media. Then suspensions of the individual microorganisms were made in bottles containing 50ml sterile peptone medium by transferring three lapfuls from the Petri dishes containing the individual microorganism into each bottle. Ten grams of each Date fruit samples were then contaminated with the selected microorganisms by immersing them in each suspensions alone for few seconds. The resulted contamination was about 106to 108cfu/g. For each date fruit sample three 10 gram portions were contaminated with the selected microorganism. One portion was treated with electrolyzed water by soaking for 5, 15 or 30 minutes, a second portion treated by soaking in sterile tap water for the same period of time, and a third portion analyzed as untreated sample. The microbial loads of the treated and untreated portions were then determined. The selected microorganisms were *S. aureus* ATCC 25923 as representative of potential pathogens, *P. aeruginosa* ATCC 27853 as representative of potential spoilage bacteria, *E. coli ATCC* 25922 as representative of coliforms, and *A. fumigatus* ATCC 204305 as a representative of potential spoilage molds.

Statistical Analysis

Analysis of variance was performed to detect differences between the microbial load of the applied treatments on samples. Duncan multiple ranges at 5% level of significance was used to compare between means.

The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 1996).

RESULTS AND DISCUSSION

Use of ozonated water to control general contamination with mesophillic aerobic bacteria, molds and yeasts

1. Treatment for 5 minutes

Contamination with mesophillic aerobic bacteria in the samples before treatment was in the range $10^2$to 10^4 cfu/g (Table 1). Soaking in sterile water for 5 minutes reduced this load by an average of 0.98 log cycles, which represent a reduction of about 88.6% of the bacterial population. Soaking in ozonated water reduced loads in the range 10^2 cfu/g to non-detectable level. Reductions in loads in the range 10^3 to 10^4 cfu/g were on average about 1.82 log cycles, i.e. an average of about 97.4% of the population was removed or an average of 0.84 log cycles over the washing effect of water. Since mesophillic aerobic bacteria are not considered an important potential spoilage agent of date fruits, their presence is only regarded as an index for the hygienic status of the fruits.

All 5 samples tested were found contaminated with molds and yeasts at loads in the range $10^2$to 10^4 cfu/g (Table 1) about 90% of which was molds. Soaking in sterile water reduced these levels of contamination by an average of 0.86 log cycles, i.e. about 83.9% of the population was removed. Soaking in ozonated water reduced contaminations in the range 10^2 cfu/g to non-detectable levels. Contaminations of the order 10^3-10^4 cfu/g, were reduced by an average of 1.69 log cycles or by 96.8%, and no yeasts were detected in the treated samples (result not shown). Molds and yeasts are considered important spoilage agents of date fruits. The Saudi standard for microbiological criteria of foods requires that the loads of yeasts in date fruits should not exceed 10 cfu/g in 3 out of 5 replicates of tested sample and that of molds not to exceed 10^2 cfu/g in 3 of 5 replicates of tested sample (SASO, 1998). This treatment reduced contamination to levels that meet Saudi standard for both molds and yeasts.

Coliforms contamination was detected in 2 of the 5 samples tested, with loads in the range $10^2$to 10^3 cfu/g. Soaking in water reduced these loads by an average of 0.84 log cycles, while soaking in ozonated water reduced contamination of the sample with 10^2 cfu/g to non-detectable level and that of the sample with 10^3 cfu/g by 1.8 log cycles (98.4%). It can therefore be concluded that contamination of date fruits with coliforms at normal levels could be controlled by this treatment.

2. Treatment for 15 minutes

The results of the 5 samples soaked in ozonated water for 15 minutes are presented in Table 2. Contamination

of the samples with mesophillic aerobic bacteria was in the normal range of 10^2 to 10^5 cfu/g. Soaking in sterile water for 15 minutes reduced these loads by an average of about 0.95 log cycles or by91.6%. Soaking in ozonated water reduced loads in the order 10^2 and 10^3 cfu/g to non-detectable levels. The loads in the order 10^4 and 10^5 cfu/g were reduced by an average of 2.29 log cycles or 99.5%, which was clearly higher than the effect of soaking in sterile water and in ozonated water for 5 minutes. This means that contamination levels of up to 10^3 cfu/g of mesophillic aerobic bacteria can be reduced to non-detectable levels by soaking in ozonated water for 15 minutes.

Contamination levels with molds and yeasts in the 5 samples tested were in the order 10^2 to 10^3 cfu/g (Table 2), again about 90% was molds. Soaking in sterile water reduced the contamination of $2.1x10^2$ cfu/g in one sample to non-detectable level, while contaminations in the other 4 samples were reduced by an average of 0.97 log cycles. Soaking in ozonated water reduced the contamination in all of the 5 samples to non-detectable levels. This treatment is therefore enough for reducing loads of molds and yeasts of up to 10^3 cfu/g to levels that make date fruits meet the Saudi standards for contamination with molds and yeasts.

Coliforms were detected in 2 of the 5 samples tested at loads of $1.4x10^2$ and $9.2x10^3$ cfu/g. Soaking in sterile water and in ozonated water reduced the load of $1.4x10^2$ cfu/g to non-detectable level, while soaking in sterile water and in ozonated water reduced the load of $9.2x10^3$ cfu/g by 1.11 and 2.07 log cycles, respectively. These results are comparable to results obtained for the other treatments discussed above and confirm that soaking in ozonated water for up to 5 minutes is quite enough to control normal levels of contamination of date fruits with coliforms.

3. Treatment for 30 minutes

The results of soaking date fruit samples in ozonated water for 30 minutes are shown in Table 3. The 5 samples were found contaminated with mesophillic aerobic bacteria at loads in the order $10^2$to 10^5 cfu/g. Soaking in sterile water for 30 minutes reduced a load of $3.0x10^2$ cfu/g in one sample to non-detectable level. The loads in the other 4 samples were reduced after soaking in water for 30 minutes by the usual values ranging between 0.84 and 1.11 log cycles. Loads of 10^2 and 10^3 cfu/g in 4 samples were reduced to non-detectable levels by soaking in the ozonated water for 30 minutes, while the load of the sample with $2.2x10^5$ cfu/g was reduced by 2.14 log cycles or about 99.3%. This effect is comparable to that reached by soaking in ozonated water for 15 minutes (2.29 log cycles for the 15 minutes soaking) which indicate that soaking for 30 minutes didn't bring about an increase in the killing effect of ozonated water. It can therefore be concluded that treatment with ozonated water for 15 minutes is the optimum treatment for the control of mesophillic aerobic bacteria in date fruits.

Molds and yeasts were found contaminating all of the 5 samples at loads in the order 10^2 to 10^4 cfu/g (Table 3). Soaking in sterile water reduced these contamination levels by an average of 0.96 log cycles or by about 88.8%, which was the usual value observed in the other treatments presented above. Soaking in the ozonated water for 30 minutes reduced the contamination in 4 samples with loads in the order 10^2 and 10^3 cfu/g to non-detectable levels. The load of the order 10^4 cfu/g was reduced by this treatment by 2.38 log cycles or by about 99.6%, with no yeasts detected in the treated samples (result not shown). This amount of reduction is enough to satisfy the Saudi standard for molds and yeasts. Anyway, soaking for 30 minutes doesn't seem to bring much more effect over soaking for 15 minutes. Since the normal level of contamination of date fruits with molds and yeasts doesn't exceed the order 10^3 cfu/g, treatment of date fruits by soaking in ozonated water for 15 minutes seem to be enough for the control of this group of microorganisms.

The picture for contamination with coliforms was as usual; only one of the 5 samples was found contaminated with this group of bacteria at a load of $4.2x10^3$ cfu/g (Table 3). Soaking in water reduced this load by 0.99 log cycles and soaking in the ozonated water reduced it to non-detectable level. It can be stressed again that contamination with coliforms is not a great problem for date fruits. Soaking in ozonated water for 15 minutes, which was found enough for the control of molds, yeasts and mesophillic aerobic bacteria, can be considered as enough for the control of contamination with coliforms.

Use of ozonated water to control contamination of date fruits with selected potential pathogenic and potential spoilage microorganisms

Table 4 contains the results of soaking 5 date fruit samples in ozonated water for 5 minutes. The levels of contamination of the untreated samples were 10^7 to 10^8 cfu/g for the bacteria and 10^6 to 10^7 cfu/g for the mold. Soaking in sterile tap water reduced the level of contamination in all tested samples by an average near one log cycle, which was the normal average value obtained for all other treatments discussed above. Soaking in ozonated water reduced the level of contamination with *S. aureus* by 1.59 to 2.11 log cycles with an average of 1.85 log cycles. In case of *P. aeruginosa*, this treatment reduced contamination by 1.22 to 1.37 log cycles with an average of 1.28 log cycles. *E. coli* was found more sensitive to treatment with ozonated water than *P. aeruginosa* but apparently less sensitive than *S. aureus*. On soaking in ozonated water for 5 minutes, contamination with this bacterium was reduced by 1.41 to

1.56 log cycles, with an average reduction of 1.49 log cycles. These results indicate that *S. aureus* was the most sensitive bacterium to treatment with ozonated water for 5 minutes, followed in sensitivity by *E. coli* and the least sensitive was *P. aeruginosa*. Treatment with ozonated water for 5 minutes reduced contamination with *A. fumigatus* by 1.83 to 2.19 log cycles with an average of about 2.0 log cycles, hence this fungus was the most sensitive to this treatment.

Results of treatments of the 4 microorganisms with ozonated water for 15 minutes are shown in Table 5. The effects of soaking in sterile tap water on the levels of contamination were similar to effects registered and discussed before. The average reduction in contamination with *S. aureus* after soaking in the ozonated water was 2.21 log cycles i.e. about 99.2%, which was more than that reached for treatment with ozonated water for 5 minutes (1.85 log cycles). Contamination of *P. aeruginosa* and *E. coli* was reduced as a result of this treatment by averages of 1.69 and 2.04 log cycles, respectively. Again the effect of this treatment on both bacteria was higher than that of treatment with ozonated water for 5 minutes. With respect to *A. fumigatus*, treatment with ozonated water for 15 minutes reduced the level of contamination by an average of 2.40 log cycles, i.e. about 99.4%. As expected the effect of this treatment was higher than that of the treatment with this water for 5 minutes.

Results of treatments with ozonated water for 30 minutes are presented in Table 6. The effect of treatment of the 4 microorganisms with ozonated water for 30 minutes was slightly higher than that of the treatment for 15 minutes (Tables 4 and 5). It was 2.30, 1.76, 2.09 and 2.53 log cycles for the 30 minutes treatment compared to 2.21, 1.69, 2.04 and 2.40 log cycles for the 15 minutes treatment for *S. aureus, P. aeruginosa, E. coli* and *A. fumigatus*, respectively.

Soaking date fruits in ozonated water for 15 minutes seems to be the most effective treatment for the control of contamination with *S. aureus,P. aeruginosa, E. coli* and *A. fumigatus*(Table 7). The effect of this treatment on the level of contamination with these microorganisms was significantly higher than that of treatment for 5 minutes but not significantly different from that of treatment for 30 minutes.

No work on treatment of date fruits with ozonated water was found in the literature cited. Xu (1999) reported >90% reduction in total bacterial count of cabbage after treatment with ozonated water for 3 minutes. According to the food Safety Network Canada (2008), *E. coli* contamination in water can be reduced by 2 log cycles after injection of 0.02 mg ozone per minute per liter. Manousaridis et al (2005) reported that ozonation (1 ppm for 60 to 90 minutes) reduced contamination of shucked mussels with aerobic mesophillic bacteria by 0.7 to 2.1 log cycles, *Pseudomonas* sp. by 0.5 to 1.1 log cycles, *Brochothrix thermosphacta* by 0.3 to 1.4 log cycles, and *Enterobacteriaceae* by 0.5 to 1.5 log cycles. Treatment with 0.12 to 3.8 ppm ozone inactivated gram-positive bacteria by 1.0 to 7.0 log cfu/ml and treatment with 0.004 to 6.5 ppm ozone reduced population of gram-negative bacteria by 0.5 to 6.5 log cfu/ml (Khadre et al. 2001). Treatment of lettuce in ozonated water (1.3 ppm) for 3 minutes reduced the load of mesophillic and psychrotrophic microorganisms by 1.2 and 1.8 log cycles, respectively (Rivera, 2005). Atia et al, (2011) found that hot air and hot water treatments of Deglate and Elak varieties date fruits reduced the number of the associated fungi (1.17, 1.17, 3.00, and 1.17 $x10^4$ colonies/g dates, respectively) compared to untreated control (12.5 and 7.84 $x10^4$ colonies/g dates). Washing date fruits with sterile water decreased numbers of fungal load (6.00 and 2.33 $x10^4$ colonies/g dates). Carnation oil treatment reduced fungal load on dates.

Conclusion

Contamination of date fruits with mesophillic aerobic bacteria was mostly in the range 10^2 to 10^4, with molds and yeasts in the range 10^2 to 10^3 and with coliforms less than 10^2 cfu/g. Treatment with ozonated water for 15 minutes reduced these levels of contamination by more than 2 log cycles which was enough to meet Saudi standard requirement. Hence normal microbial contamination of date fruits can be controlled by treatment with ozonated water.

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Tables

Table 1: Effect of soaking in tap water and ozonated water for 5 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$3.2x10^4$		$4.9x10^2$		n.d.	
1 (T)	$4.0x10^3$	0.91	79	0.79	n.d.	-
1 (O)	$5.2x10^2$	1.79	n.d.	-	n.d.	-
2 (U)	$7.7x10^2$		$2.7x10^4$		$5.3x10^3$	
2 (T)	$1.2x10^2$	0.81	$3.6x10^3$	0.87	$7.4x10^2$	0.85
2 (O)	n.d.	-	$2.8x10^2$	1.98	84	1.83
3 (U)	$7.0x10^3$		$8.1x10^2$		n.d.	
3 (T)	$8.5x10^2$	0.92	$1.4x10^2$	0.76	n.d.	-
3 (O)	$1.1x10^2$	1.81	n.d.	-	n.d.	-
4 (U)	$1.5x10^4$		$6.5x10^3$		$6.8x10^2$	
4 (T)	$9.7x10^2$	1.19	$4.6x10^2$	1.15	$1.0x10^2$	0.83
4 (O)	$2.5x10^2$	1.78	75	1.93	n.d.	-
5 (U)	$6.2x10^3$		$1.4x10^3$		n.d.	
5 (T)	$5.0x10^2$	1.09	$2.6x10^2$	0.74	n.d.	-

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
5 (O)	80	1.89	$1.0x10^2$	1.15	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 2: Effect of soaking in tap water and ozonated water for 15 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$4.4x10^5$		$2.1x10^2$		$9.2x10^3$	
1 (T)	$3.7x10^4$	1.07	n.d.	2.32	$7.0x10^2$	1.11
1 (O)	$2.6x10^3$	2.23	n.d.	2.32	77	2.07
2 (U)	$5.1x10^3$		$8.4x10^2$		n.d.	
2 (T)	$6.6x10^2$	0.89	83	1.00	n.d.	-
2 (O)	n.d.	-	n.d.	2.92	n.d.	-
3 (U)	$4.1x10^2$		$1.9x10^3$		n.d.	
3 (T)	67	0.78	$2.4x10^2$	0.90	n.d.	-
3 (O)	n.d.	-	n.d.	3.28	n.d.	-
4 (U)	$5.5x10^3$		$7.0x10^2$		$1.4x10^2$	
4 (T)	$6.0x10^2$	0.96	64	1.04	n.d.	2.15
4 (O)	n.d.	-	n.d.	2.85	n.d.	2.15
5 (U)	$2.2x10^4$		$4.7x10^3$		n.d.	
5 (T)	$2.0x10^3$	1.04	$5.3x10^2$	0.95	n.d.	-
5 (O)	$1.0x10^2$	2.34	n.d.	3.67	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 3: Effect of soaking in tap water and ozonated water for 30 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$3.0x10^2$		$4.0x10^3$		n.d.	
1 (T)	n.d.	-	$5.8x10^2$	0.84	n.d.	-
1 (O)	n.d.	-	n.d.	3.60	n.d.	-

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
2 (U)	$4.8x10^3$		$5.5x10^4$		n.d.	
2 (T)	$3.7x10^2$	1.11	$4.1x10^3$	1.13	n.d.	-
2 (O)	n.d.	-	$2.3x10^2$	2.38	n.d.	-
3 (U)	$2.2x10^5$		$7.4x10^2$		n.d.	
3 (T)	$1.8x10^4$	1.08	90	0.92	n.d.	-
3 (O)	$1.6x10^3$	2.14	n.d.	2.87	n.d.	-
4 (U)	$5.3x10^3$		$6.3x10^2$		$4.2x10^3$	
4 (T)	$7.5x10^2$	0.84	66	0.98	$4.3x10^2$	0.99
4 (O)	n.d.	-	n.d.	2.80	n.d.	3.62
5 (U)	$7.1x10^2$		$8.0x10^3$		n.d.	
5 (T)	81	0.94	$9.1x10^2$	0.94	n.d.	-
5 (O)	n.d.	-	n.d.	3.90	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 4: Effect of soaking in tap water and ozonated water for 5 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
1 (U)	$3.5x10^8$		$1.5x10^8$		$1.6x10^7$		$5.5x10^6$	
1 (T)	$4.4x10^7$	0.90	$3.1x10^7$	0.69	$2.4x10^6$	0.82	$3.0x10^5$	1.26
1 (O)	$5.3x10^6$	1.82	$8.7x10^6$	1.24	$4.4x10^5$	1.56	$6.5x10^4$	1.83
2 (U)	$6.5x10^7$		$7.5x10^7$		$2.1x10^8$		$6.9x10^6$	
2 (T)	$7.2x10^6$	0.95	$8.1x10^6$	0.97	$3.5x10^7$	0.78	$7.6x10^5$	0.96
2 (O)	$8.2x10^5$	1.90	$3.2x10^6$	1.37	$7.0x10^6$	1.47	$8.0x10^4$	1.94
3 (U)	$2.2x10^8$		$2.0x10^8$		$1.3x10^8$		$3.0x10^7$	
3 (T)	$2.8x10^7$	0.89	$3.5x10^7$	0.76	$3.0x10^7$	0.63	$4.2x10^6$	0.86
3 (O)	$5.6x10^6$	1.59	$1.2x10^7$	1.22	$5.0x10^6$	1.41	$3.4x10^5$	1.95

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
4 (U)	$5.8x10^7$		$6.3x10^7$		$4.2x10^7$		$3.2x10^7$	
4 (T)	$7.5x10^6$	0.88	$7.4x10^6$	0.93	$5.3x10^6$	0.90	$4.3x10^6$	0.88
4 (O)	$8.2x10^5$	1.85	$3.2x10^6$	1.29	$1.3x10^6$	1.51	$2.1x10^5$	2.19
5 (U)	$7.1x10^7$		$8.0x10^7$		$2.2x10^8$		$8.1x10^6$	
5 (T)	$8.4x10^6$	0.93	$9.1x10^6$	0.94	$3.3x10^7$	0.82	$9.0x10^5$	0.96
5 (O)	5.5x`0^5	2.11	$4.3x10^6$	1.27	$7.0x10^6$	1.49	$8.3x10^4$	1.99

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 5: Effect of soaking in tap water and ozonated water for 15 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* cfu/g)	Reduction (log cycles)
1 (U)	$7.4x10^7$		$3.0x10^8$		$3.1x10^7$		$7.5x10^6$	
1 (T)	$6.8x10^6$	1.04	$4.5x10^7$	0.83	$4.2x10^6$	0.87	$6.7x10^5$	1.05
1 (O)	$5.0x10^5$	2.14	$4.6x10^6$	1.82	$2.3x10^5$	2.13	$4.5x10^4$	2.23
2 (U)	$2.4x10^8$		$8.8x10^7$		$8.2x10^7$		$1.8x10^7$	
2 (T)	$3.2x10^7$	0.87	$1.1x10^7$	0.90	$8.5x10^6$	0.98	$9.0x10^5$	1.31
2 (O)	$9.7x10^5$	2.39	$9.2x10^5$	1.98	$6.6x10^5$	2.09	$7.4x10^4$	2.39
3 (U)	$2.6x10^8$		$3.3x10^8$		$4.0x10^8$		$5.1x10^7$	
3 (T)	$1.7x10^7$	1.18	$4.0x10^7$	0.92	$5.9x10^7$	0.83	$4.6x10^6$	1.05
3 (O)	$1.5x10^6$	2.23	$4.7x10^6$	1.85	$3.0x10^6$	2.12	$2.0x10^5$	2.41
4 (U)	$7.2x10^7$		$1.4x10^8$		$6.3x10^7$		$6.2x10^6$	
4 (T)	$7.5x10^6$	0.94	$8.7x10^6$	1.21	$5.6x10^6$	1.05	$7.3x10^5$	0.93
4 (O)	$4.0x10^5$	2.22	$3.8x10^6$	1.57	$7.2x10^5$	1.94	$4.0x10^4$	2.19
5 (U)	$3.1x10^8$		$8.6x10^7$		$3.2x10^8$		$3.0x10^7$	
5 (T)	$4.4x10^7$	0.85	$7.7x10^6$	1.04	$4.0x10^7$	0.91	$4.2x10^6$	0.86
5 (O)	2.5x`0^6	2.09	$5.2x10^6$	1.21	$4.1x10^6$	1.90	$5.1x10^4$	2.77

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 6: Effect of soaking in tap water and ozonated water for 30 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
1 (U)	$2.4x10^8$		$1.2x10^8$		$4.0x10^8$		$1.7x10^7$	
1 (T)	$3.0x10^7$	0.90	$3.0x10^7$	0.63	$5.2x10^7$	0.88	$2.1x10^6$	0.91
1 (O)	$1.6x10^6$	2.18	$2.2x10^6$	1.74	$3.3x10^6$	2.08	$6.4x10^4$	2.43
2 (U)	$4.1x10^8$		$7.9x10^7$		$7.6x10^7$		$5.9x10^6$	
2 (T)	$3.8x10^7$	1.03	$8.0x10^6$	1.00	$6.7x10^6$	1.05	$5.2x10^5$	1.05
2 (O)	$2.5x10^6$	2.21	$2.3x10^6$	1.54	$5.5x10^5$	2.14	$1.3x10^4$	2.66
3 (U)	$7.8x10^7$		$6.8x10^7$		$3.4x10^8$		$8.0x10^6$	
3 (T)	$8.2x10^6$	0.98	$5.7x10^6$	1.07	$2.2x10^7$	1.19	$6.8x10^5$	1.07
3 (O)	$2.1x10^5$	2.57	$8.7x10^5$	1.89	$2.7x10^6$	2.10	$5.3x10^4$	2.18
4 (U)	$9.0x10^7$		$7.0x10^7$		$2.9x10^8$		$7.5x10^6$	
4 (T)	$8.8x10^6$	1.01	$7.6x10^6$	0.97	$3.2x10^7$	0.95	$6.7x10^5$	1.05
4 (O)	$5.3x10^5$	2.22	$8.0x10^5$	1.95	$4.0x10^6$	1.86	$2.3x10^4$	2.52
5 (U)	$2.6x10^8$		$2.6x10^8$		$6.0x10^7$		$2.5x10^7$	
5 (T)	$3.0x10^7$	0.93	$3.5x10^7$	0.87	$5.8x10^6$	1.02	$3.2x10^6$	0.89
5 (O)	$1.2x10^6$	2.33	$5.3x10^6$	1.69	$3.1x10^5$	2.29	$3.6x10^4$	2.84

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 7: Effect of soaking in ozonated water for 5, 15 and 30 minutes on contamination of date fruit samples with some microorganisms

Time (minutes)	Amount of reduction (log cycles)			
	S. aureus	*P. aeruginosa*	*E. coli*	*A. fumigatus*
5	1.85^b	1.27^b	1.48^b	2.00^b
15	2.24^a	1.68^a	2.03^a	2.38^a
30	2.32^a	1.76^a	2.09^a	2.52^a

Means within columns having same letter are not significantly different ($P \leq 0.05$)

Isolation and identification of fungal pathogen from *Phoenix dactylifera* (date palm) tissues in Eltaweel oasis in Northern Kordofan, Sudan

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ABSTRACT

Fusarium oxysporum and Fusarium solani were isolated following standard methods from all infected parts of diseased Date palm (*Phoenix dactylifera*) leaves. The pathogenicity tests on three weeks offshoot proved that F. oxysporum and F. solani were the most destructive fungus on leaves. Old leaves at lower part susceptible to fusarium wilt. Total discolouration of leaflet partial or on one side of the rachis was noticed. Desiccation or death was found to be associated with a shade of brown in one site leaflets whereas the opposite sites of the rachis were healthy.

Keywords: *Fusarium*, micronidia, macrconidia, chlamydospores, leaves.

INTRODUCTION

Fusarium spp. are Hypocreales Ascomycota (Moretti, 2009; Davis *et al.,* 2010).The genus *Fusarium* comprises a high number of fungal species that causes diseases in several agriculturally important crops (Garofalo *et al.,* 2003). Many of *Fusarium* species producing a wide range of biologically active secondary metabolites (e.g. mycotoxins) with extraordinary chemical diversity (Moretti, 2009; Davis *et al.,* 2010; Garofalo *et al.,* 2003).

Infections of date palm by *Fusarium* can occur from germinating seeds up to mature vegetative tissues, depending on the host plant and *Fusarium* species involved (Elliott, 2006; El-Deeb *et al.,* 2006; Armengol *et al.,* 2005). Therefore, it has to be identified accurately and as early as possible to predict the potential toxicological risk to date palm trees, and to prevent toxins entering the food chain (Elliott, 2006; El-Deeb *et al.,* 2006; Armengol *et al.,* 2005).

MATERIAL AND METHODS

The infected wooden parts were collected for identification of the fungus damage. The standard techniques set by Armengol *et al.,* (2005) were followed for isolation and identification of fungal pathogen from date palm.

1. Sterilization of glass ware and steel tools

Petri dishes, forceps, scalpels, scissors were sterilized by using hot air oven at 180 ˚C for one hour. These tools were kept sterilized until used.

2. Preparation of culture media for growing of fungi

Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA), HiMedia laboratories Pvt. Ltd India, were used for cultivation and isolation of pathogenic fungi from the date palm leaves with black spots due to fungal infection. The first culture medium (PDA), (HiMedia laboratories Pvt. Ltd, India) was prepared by dissolving 39 g of medium powder in 1000 ml distilled water. The mixture was boiled in a

water bath to melt the agar at 100 ˚C for 15 minutes. Then the flask containing the rehydrated medium was plugged properly with cotton wool and sterilized by autoclaving at 15 pound pressure for 20 min. After autoclaving the sterilized medium was cooled and kept at 45 ˚C in a water bath, and then it was poured into sterilized petri plates under aseptic conditions in a laminar flow beside Bunsen burner flame. Each plate contained about 15-20 ml of liquid agar; plates were allowed to solidify at room temperature and kept sterilized at 4 ˚C until used. The second culture medium was (SDA), (HiMedia laboratories Pvt. Ltd, India). It was prepared by the same procedure described above and medium plates were kept sterilized at 4 ˚C until used.

3. Collection of samples

Discoloured dead leaves of date palm with black spots on surface (Fig. 1) suspected to be infected with fungal pathogen were collected randomly from different date palm trees in Northern Kordofan state, Bara Province. Collected samples were brought to the laboratory in sterilized box and kept dry at room temperature until used.

4. Isolation of fungi from Date Palm leaves tissues

Collected leaves samples were cut into small pieces with sterile scissor. Surface sterilization was done to remove surface contaminants spores by washing in 95 % ethanol for one minute. After alcohol washing the pieces of leaves were transferred by sterile forceps into petri dishes contained sterile deionized water and washed gently three times after that the pieces were placed on PDA and others on SDA. The cultured plates were incubated at 25˚C for 4 days after the end of which the fungus growth was noticed Figs. 2.

5. Purification of isolated fungal colonies

Pure cultures were obtained from the mixed isolated colonies by picking up a part from each colony using sterile forceps and transferred into new petri plates containing PDA medium. Nine colonies have been sub cultured and the plates were incubated at 25 ˚C for 4 days after which isolated fungal colonies were ready for identification studies. The identification was done on the basis of morphological and cultural features following Ellis *et al.,* (2007).

6. Microscopic examinations for isolated fungi

Wet mounting preparations from each colony were made by removing a small piece of hyphae using sterile needle and mixed with a drop of lacto phenol cotton blue dye on glass slide. A cover slip was applied over each slide before examined under compound microscope. Another wet mounting technique was used. In this technique adhesive plaster method, a piece of adhesive plaster was attached above the mycelia colonies removing some fungal structure, then applied to slide on which lacto phenol cotton blue was dropped. Slides were examined under microscope. Macro and micro conidia characteristics of the hyphae, presence or absence of septation and the shapes of spores were recorded for identification of the isolates.

7. Re-infecting the date palm tissues with isolated fungi

An experiment was applied in order to confirm the pathogenicity of the isolated fungi. Selected date palm trees in the yard of Faculty of Science, University of Khartoum were inoculated with isolated fungi. Inoculation was done by cleaning and disinfecting surface of young green leaves with 70 % ethanol. Small area in the middle of the leave was scratched by sterile scalpel. A small part from each colony was removed by sterile forceps and inoculated onto scratched areas and covered with sterile foil. The whole inoculated leaves were covered with wet plastic sack sealed to leaflets with adhesive plaster to maintain moisture inside the inoculums. The infected leaves were left for three weeks for developing fungal pathogen as recommended by Koch's postulates.

RESULTS

In this study several date palm trees in the oases showed symptoms of a fungal disease caused by Fusarium wilt or vascular wilt and there are two species of *Fusarium* responsible for disease such as: *Fusarium oxysporum* and *Fusarium solani.*

Old leaves at lower part are susceptible to Fusarium wilt. Total discoloration of Leaflets partial or on one side of the rachis was noticed.

Desiccation or death was found to be associated with a shade of brown in one site leaflets whereas the opposite sites of the rachis were healthy.

1. Macroscopic characters

The major macroscopic morphological characteristics including growth time, colonies colours and sporulation conditions were recorded and used in identification of the isolates in the present study. Colonies growth on (Potato dextrose agar) PDA medium was obtained after 4 days incubation at 25 ˚C. Nine colonies were grown; they showed different colours that vary from white, pink, grey, green-purple and beige on obverse side. Colours were most orange and dark grey on the reverse side. The morphological characteristics of the isolates are presented in Table (1) and Fig. 3.

2. Microscopic characters:

Microscopic examination was applied to isolate by mounting a piece of hyphae with a drop of lactopphenol cotton blue on slide and covered with slips and examined under microscope. Results of examination revealed that most observed features were septation of the hyphae in all isolated colonies and formation of macro and micro conidia spores as shown in Fig. 4.

In some isolates chlamydosopres with curved spindle shape were found in Fig .5. This is one of unique characteristics of *Fusarium* spp. identification. The isolates features in the present study matched features of two Fusarium species previously described and displayed by (Ellis *et al.,* 2007). Also similar evidences are offered by the web site www. mycologyonline, where the fungus species is supported with photos of the septated hyphae, colours of colonies, shape of macro and micronidia and chlamydospores.

The morphological features of *Fusarium oxysporum* indicated while coloured, macroconidia is fusiform slightly curved and pointed at the tip mostly three septate. Microconidia are abundant, not in chains, ellipsoidal to cylindrical, straight or often curved. The morphological features of *Fusarium solani* showed rapid growth of the colonies in less than 4 days. Macroconidia are formed from multi-branched conidiophores. With 3- to 5 septate, fusiform often moderately curved. Micro conidia are usually abundant, cylindrical to oval. Chlamydosopres are hyaline globes, borne in single or in pairs on short lateral hyphen branches.

DISCUSSION

The present study, found that *Fusarium oxysporum* and *Fusarium solani* are the causative agents affecting date palm trees in oases of Northern Kordofan. These results agree with Logan and El Bakri (1990) who isolated the same genus *Fusarium moniliforme* from Dongola State. The observed symptoms on the surveyed date palm trees in the study area included the appearance of black spots and scales on the leaves surfaces which indicated that fungal pathogen was the cause agent of disease case. The isolated pathogenic features in the present study, matched the features of two *Fusarium* species previously described by (Ellis *et al.,* 2007). Confirmatory evidences were the similarity of the septated hyphae, colours of colonies, shape of micronidia and macrconidia and the shapes of chlamydospores, with those published by the web site www.mycologyonline.

Morphological features of *F. oxysporum* isolates indicated that they grow rapidly in 4 days, its colonies are white sized about 4.5 cm in diameter, macroconidia is fusiform slightly curved and pointed at the tip mostly three septate, microconidia are abundant, not in chains, ellipsoidal to cylindrical, straight or often curved. This is in accord with Garofalo and McMillan, 2003; Armengol *et al.,* 2005; El Deeb *et al.,* 2006; Moretti, 2009; and Ammar and El-Naggar, 2011. Colonies of *F. solani* grow rapidly in 4 days, its macroconidia are formed from multi-branched conidiophores with 3 to 5 septate, fusiform and often moderately curved. The Microconidia are usually abundant, cylindrical to oval. Chlamydosopres are hyaline globes, borne in single or in pairs on short lateral hyphen branches. The morphological features recorded during this study are in accord with (Armengol *et al.,* 2005; El Deeb *et al.,* 2006 and Moretti, 2009). Several fungal diseases of date palm trees have been reported from many date producing countries but *F. oxysporum* and *F. solani* are the most serious fungi causing diseases to the trees (El Deeb *et al.,* 2006).

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www.mycologyonline

Table. 1. Morphological features of the isolated fungi

Colour on		Size of colony (cm)	Shape of spores	No. of septation Spores
Obverse	Reverse			
Pink	Orange	4	Ovid	0
Pink with green ring	Orange	4	Ovid	0
Grey	Dark purple	6.5	Ovid	0
White	Bright brown	6	Ovid + fusiform	0
Dark green	Black	5.5	Ovid	0
Pale pink	Dark pink	3.5	Ovid	0
White pinkish	Orange	4.2	Fusiform	3
Creamy-pink	Orange	2.8	Ovid	0
Light brown	Dark orange	5.4	Ovid	0

Figures

Fig.1. Discoloured dead leaves of a date palm with black spots of *Fusarium* sp.

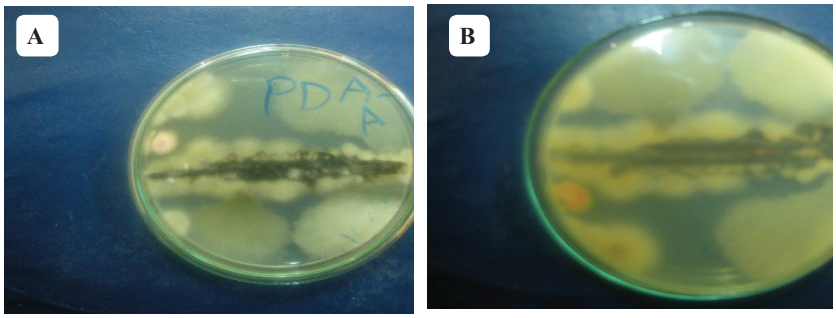

Fig.2. Fungus growth after 4 days incubation at 25˚C. Obverse side (A), reverse side (B).

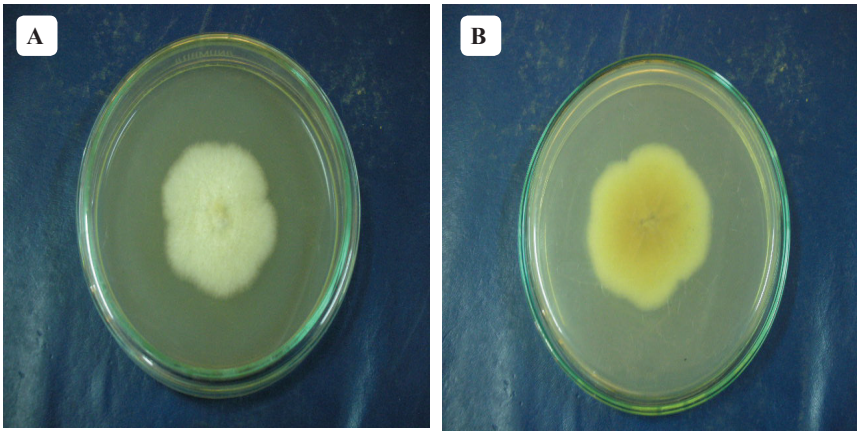

Fig. 3. Morphological characters of isolated fungi obverse side (A) white colour and Reverse side with yellowish colour (B).

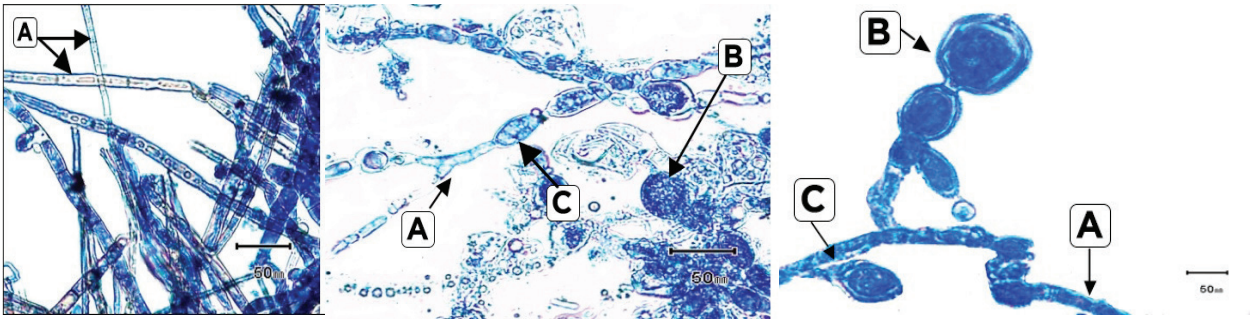

Fig . 4. Formation of mycila (A), macro-conidia (B) and micro-conidia (C) form of *Fusarium* sp.

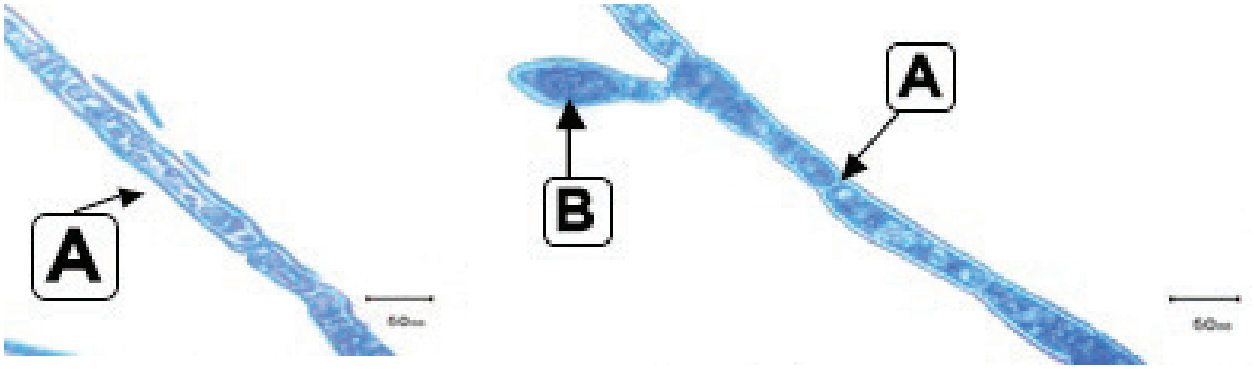

Fig . 5. Formation of mycila (A) and chlamydo spores (B) with curved spindle shape.

Date Palm Processing and Marketing

Analysis of phenolic compounds extracted from date of *Phoenix dactylifera* L (cultivar:Deglet Nour). search analgesic activity.

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ABSTRACT

The date palm is a species particularly adapted to arid lands. It represents for the people of the Saharan oases indispensable species as it constitutes their resource base, especially for its fruit, date.In our work we were interested in extract, separation, purification and identification of polyphenols contained in the date of Poenix dactylifera L (cultivar:Deglet nour) of Adrar. The study of analgesic activity of these compounds in a second time. The extraction of large families of polyphenols, with different solvents (polar and non polar),allowed us to:-quantify by spectrophotometry UV-visible following compounds (flavones, flavonols: 1,12 mg / g ± 0.14, anthocyanins: 1,75 mg / g ± 0,43, C-glycosides: 2,286 mg / g ± 0,29, and total phenols :3,6%) .Reveal and identify by thin layer chromatography (TLC), paper (CP), and high performance liquid chromatography (HPLC), the composition of each family. We have detected on HPLC profiles following compounds: as Flavone: Luteolin, Tricin, Chrysoeriol and 2 Flavonols: Quercetin, Isorhamnetin .The Cyanidin is the only anthocyanin isolated .The evaluation of analgesic activity occurs by injection of acetic acid intraperitoneally in mice that causes a painfulreaction manifested by cramping, which can be reduced by an analgesic product. This study compares the reduction in the number of cramps after administration of doses of test and reference product (Aspirin).Flavone C-glycosides and Anthocyanin extract of the date present a better analgesic activity than aspirin reference. The percentage of protection obtained from the results of "Cramping test" shows a significant activity of both extracts with the value (66.3%), which is double that obtained for the reference analgesic product (32.5%). The extract date is not only rich in phenolic compounds but they have a high analgesic activity.

Key words : date palm, date, flavonoids, sahara, cramps, analgesic

INTRODUCTION

The date palm or *Phoenix dactylifera* L. is the only species of the genus to be able to adapt to the hottest and driest regions in the world. At the base of the diet of saharan people the date palm is probably for agriculture in arids lands the best source (figure1).

In Algeria palm grove covers about 1 million hectares, almost all of the agricultural land areas located below the isohyet 100 mm / year(Sahki and Sahki 2004).

The number of palm is estimated at 17 million trees. It is the pillar of oasis agriculture and contributes for a large part to maintain the oasis biodiversity. A local and regional research in the field of flavonic chemistry has a certain

importance. It focused on the isolation and identification of the metabolic pathways of natural compounds that are of major importance in the biochemical mechanisms involved in the nutritional value of fruit: the date.

It is widely used by the local population as its therapeutic virtues.It is in this context that our work, is related, on the identification of the analgesic activity of flavonic content of fruit.This work is the first in Algeria.

MATERIALS AND METHODS

Plant material

The study was performed on 22 individuals of date from cultivar Deglet Nour harvested in the Biskra region (South-eastern Algeria) in 2012. The harvested plant material was kept cold and protected from light.

Animal material

The pharmacological test was performed on 40 albino mice (*Mus musculus*) having a weight of 20g ± 5.

Extraction of flavonoid aglycones

The method used was developed by Lebreton in 1967. It consists of hot acid hydrolysis (2N HCl) of cut plant material for 40 minutes in a water bath at 40°c. This hydrolysis allows the transformation of leucoanthocyanins to the corresponding anthocyanin and flavonoid aglycones released from their O-glycosides.

Extraction of glycosides

This technique was developed in 1973 by Harborne 1973. This is a maceration of plant material in a hydroalcolique solution (70:30) to extract glycosids (O-glycosides and C-glycosides).

Spectrophotometric analysis

The quantitative evaluation of phenolic compounds (flavonols, flavones, anthocyanins and Glycosides) of 22 samples of dates is based on UV-visible spectrophotometric assay at 430 nm and 520nm.

Qualitative analysis by HPLC isocratic and gradient with solvents H ² 0 – acetonitrile and methanol - acetic acid depending on the type of compounds (flavone aglycons, O- glycosides or anthocyanins).

Several chromatographic methods were used for the analysis of our extracts. The thin layer chromatography, high performance liquid chromatography, and UV-Visible spectrophotometry (Markham 1982).

Analgesic test

It consists of an evaluation of the analgesic activity of extracts of date palm dates by the technique of «Cramping test»(Vogel and Vogel, 1997).

After administration of the acetic acid to cause a painful reaction manifested by torsional movements of the abdomen with stretching of the hind legs (cramps).

The animals were fasted the night before the test. Thereafter, they receive through-gastric respectively 0.5mL of saline; reference product Feldene (20mg) and plant extracts (anthocyanins, glycosides and C-glycosides).

After 30 minutes, were injected intraperitoneally with 0.2mL of acetic acid 1% per mouse. After 5 minutes, counting cramps is achieved by direct observation for 10 minutes.

RESULTS AND DISCUSSIONS

The results obtained relate of the one part the biochemical study and biological activity of the other.

Results of the phytochemical analysis of date extracts

The extraction of large families of polyphenols, with different solvents (polar and non polar),allowed us to:

- Quantify by spectrophotometry UV-visible following compounds (flavones- flavonols: 1.12 mg / g ± 0.04, anthocyanins: 1.75 mg / g ± 0,065 C-glycosids: 1.08 mg / g ± 0,029, and hétèrosides 2.28mg/g±0,176) .Total phenols 3.6%.
- Reveal and identify by thin layer chromatography (TLC), paper (CP), and high performance liquid chromatography (HPLC), the composition of each family. . We have detected on HPLC profiles following compounds: as Flavone: Luteolin, Tricin, Chrysoeriol and 2 Flavonols: Quercetin, Isorhamnetin .The Cyanidin is the only anthocyanin isolated . We found the same compounds that we isolated from date palm leaflets (Ouafi 2007, Ouafi and Bounaga 2010)

Results analgesic activity

The mean number of cramps are calculated for each batch. The results obtained are summarized in the table 1.

After counting cramps the percentage protection(figure2) is calculated for each batch as follows:

Calculation of% protection :

$$\% \text{ of protection} = \frac{\text{Mean value of cramps of control beach - Mean value of cramps of E beach}}{\text{Mean value of cramps of control beach}}$$

According to the results, we note that the coefficient of protection of the reference product (Aspirin) is 32.5% meaning that the mice developed a more or less normal in reaction to the pain response.

By comparing the different results, we note that controls have a very low percentage of protection (0%) compared to the reference and testing. Which highlights the role of the drug used in reducing pain manifested by fewer cramps. The two test batches each have the same coverage rate of 66.3% for anthocyanins and heterosides.

1.Comparison between the percentages of protection : we set the null hypothesis H0: there is no significant difference between plant extracts and the reference product keywords.

We calculate two standard deviations: Since the percentage of equivalent protection for both extracts (butanol and ether) have a standard common type is calculated.

ε 9.6 > 1.96: The H0 hypothesis is rejected, so there is indeed a significant difference between plant extracts and the reference product.

The use of Student's test, enabled us to conclude that the two plant extracts are significantly more effective than aspirin.

2. Discussion: the analgesic activity of polyphenolic extracts could be due to flavonoids. This can be explained by the ability of these to inhibit prostaglandins. These sensitize peripheral pain receptors to the action algogenic of other mediators (histamine and bradykinin). Blocking their synthesis will remove the effects of sensitization and reduces pain (Chauvelot-Moachon, 1988).

We can say that the phenolic compounds of the date of date palm tree have a strong analgesic activity which explains its use to relieve abdominal pain by the population of the southern regions of Algeria. (Cheriti et al. 2000).

CONCLUSION

Quantitative analysis by spectrophotometry UV-Visible of the date extract of date palm tree of Biskra, allowed us to evaluate the mean values of various phenolic compounds (flavones, flavonols, anthocyanins, glycosides and C-glycosides flavone.

Finally, the analgesic activity of the date extract of cultivar Deglet Noor has been demonstrated so evident and statistically valid. It is due to anthocyanins and heterosides

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Table

Table 1. Results count cramps for each batch

Number cramps / Mouse	Controls	Reference (Aspirin)	Anthocyanins -C-glycosids (Test 1)	Heterosids (Test 2)
Mean value	21± 3,90	10,8± 0,6	5,4 ± 0,3	5,5± 0,3
% of protection	0 %	32,5%	66,3%	66,3%

Figures

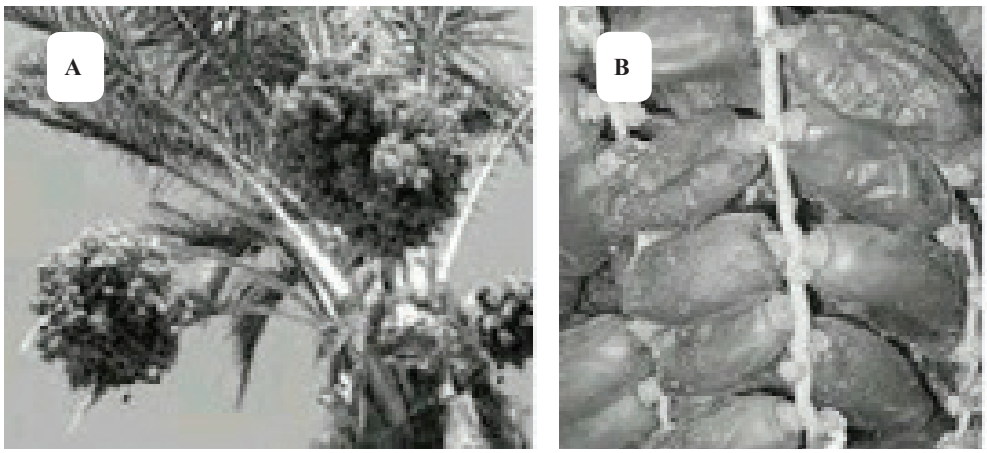

Fig .1. Date palm tree (A) and dates of cultivar Deglet Nour(B).

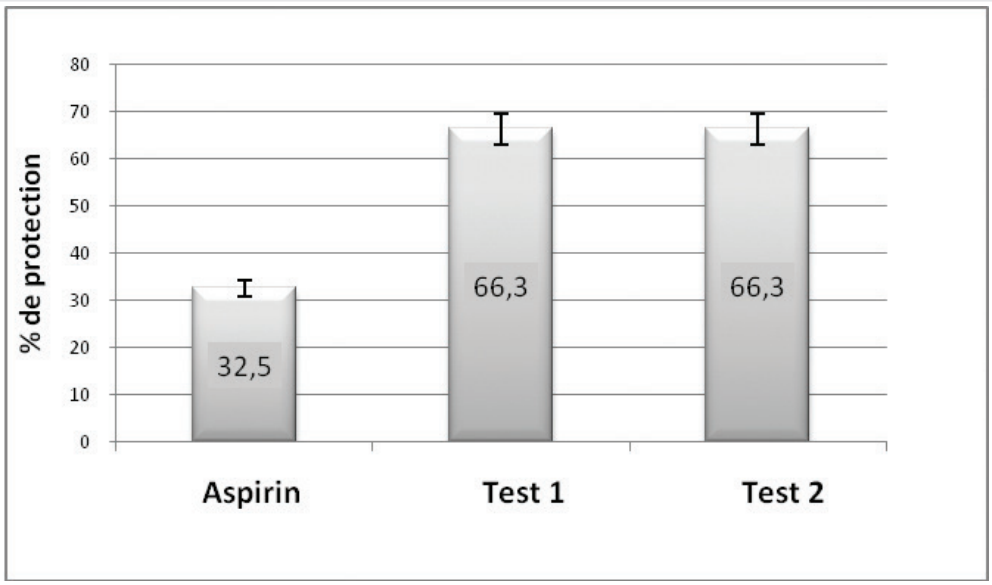

Fig. 2. Histogram showing the percentages of protection of the three test batches

Multi-period dynamic programming analysis determining the optimal replanting age of date palm

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ABSTRACT

The government of Oman has implemented a program to rejuvenate the sector by planting 1 million date palms. Date palm could be planted as new-plantings requiring additional land, water and other resources and/or be replanted substituting aged and unproductive palms without additional commitment the resources. Replanting of perennial crops is an agronomic practice that maximizes and sustains long-term benefits. Although the optimal age of replanting of several perennial crops have been scientifically estimated, the optimal age to replant date palm has not yet been scientifically examined. This study estimated the optimal age to replant date palm. Two alternative analytical models were used to estimate the optimal age of replanting of date palm, namely; Comparison of Equivalent Annual Net Revenue (CEAN) and Multi-Period Dynamic Linear Programming Model (MPDLP). Solution procedures of both models are based on the theory of optimal replacement of capital assets. Data on date palm age-yield relationship and other socio-economic variables were gleaned through a farm survey of 34 large commercial farms, in the Al-Dakhilya governorate. The study estimated the optimal age of replanting date palm as 50-55 years. The optimal age to replant date palm was sensitive only to changes in the interest rates. Low interest rates shortened the optimal age of date palm replanting. The study derived the optimal replanting schedule for date palm for Oman. The incremental revenue to Oman through replanting was estimated to be 7 million OR/ year.

Key words: Interest rates, replacement of capital assets, perennial crops, Sultanate of Oman.

INTRODUCTION

Date palm, which is a perennial crop is culturally, socially and economically the most important fruit crop in the Sultanate of Oman. The Sultanate is ranked ninth in world date *(Phoenix dactylifera L.)* production with a production of 255,871 tons, which represents 3.63% of the world production (FAOSTAT, 2010). Since year 2000 date production has stagnated and/ or declined. Some factors that have contributed to the reduction of date production are the non-availability of skilled labor to carry out field operations, occurrence of pests and diseases, harvest and post-harvest loses and degradation of soil and water quality (Al-Yahyai, 2007). The government of Oman through a decree by His Majesty the Sultan of Oman has embarked on a program of planting one million date palm trees, to rejuvenate the date sector. The planting of date palms could either be on new lands, or be replanting of old unproductive date palms in existing agricultural lands. Replanting of unproductive old plants is an agronomic practice in perennial crop management to maximize and sustain income overtime. Further replanting does not require substantial incremental resources than presently committed. This is particularly important in the Sultanate of Oman where water is extremely scarce. Replanting of date palm provides an opportunity to use the presently committed resources and improve farms towards economic production systems, which will improve the livelihood of farm dependents and increase the contribution to nation's national income. However the agronomic practice of replanting of date palm has not been formally adopted and promoted in the Sultanate of Oman.

Though research literature is replete with methodologies to estimate the optimal replanting age for many other perennial crops, there is no reported research on estimation of the optimal age for replanting of date palm. An estimate of the optimal age of replanting of date palm could contribute in the short-term to the replanting of date palms through one million date palm project and in the long-term the method of analysis and the estimate of optimal replanting age could be adopted by extension services to guide farmers to improve and sustain farm incomes and livelihoods. Replanting of date palm could maximize and sustain income from farming. The objectives of this study are as follow.

1. Estimate the optimal age to replant date palm, through two economic models [Comparison of Equivalent Annual Net Revenue (CEAN) model and Multi-Period Linear Programming Model (MPLP)].
2. Estimate the incremental income that could be obtained through replanting of date palm.
3. Recommend a schedule of optimal age-replant date palm in existing farms to improve income in the Sultanate of Oman.

The earliest and widely quoted research source on proposing analytical techniques to determine optimum replacement age of assets is by (Faris, 1960). The paper presents decisions rules that could be followed in deciding the replacements of assets that have: a short production period with revenue being realized by the sale of the asset (ex: buying and selling feeder cattle); a long production period with revenue being realized by the sale of the asset (ex: forestry); a long production period with revenues being realized throughout the life of the asset (ex: orchards; perennial crops including date palms). The rule of replanting for such crop assets as (Faris, 1960) derives is that the optimum replanting age is when the annual net revenue from "present" trees is equal to the anticipated amortized present value of the net revenue from the "future" trees. (Perrin, 1972) has through mathematical derivations clearly derived and confirmed on continuous time scenario the principles of decision making on asset replacement that was proposed by (Faris, 1960) on discrete scenario.

Analytical methods

Two alternatives analytical methods, using the principles of decision making on asset replacement have been developed and used in this study to estimate the optimal replanting age of date palm. These methods are referred to as Comparison of Equivalent Annual Net revenues (CEAN) and Multi-Period Dynamic Linear Programming (MPDLP) model. The analysis of CEAN model is restricted to data related to a single palm whilst the MPDLP model could analyze a date palm farm with palms of different ages.

Comparison of Equivalent Annual Net Revenues (CEAN) Model

The principles on decision making of asset replacement that were proposed by (Faris, 1960) have been mathematically elucidated by (Perrin, 1972). (Etherington, 1977) has applied (Perrin's, 1972) mathematical exposition on principles on decision making of asset replacement, to analyses decision making of replanting rubber trees. This study has adopted (Etherington's, 1977) exposition to explain the economic principles of deciding the age of replanting of date palm.

Multi-Period Dynamic Linear Programming (MPDLP) Model

Agricultural decision making of particularly perennial crops is characterized with multiple year dynamics, where a present activity/decisions influence future activity/decisions (McCarl and Spreen, 1997). In this study the decision to replant or (not-replant) date palm in a given year (i.e. the time path of replanting) has an influence on the future flow of net revenue from date palm. The easiest way of developing models that incorporate time is to extend the linear models developed from single period models. Using this format, multi-period models can be thought of as a series of single-period models linked by dynamic constraints or "equations of motion" that link the periods. (MPDLP) Models are widely known and used in agricultural systems modeling (Romero and Rehman, 1989). MPDLP models recognize inter temporal linkages in farm activities and maximizes the net present value of profit (or an appropriate objective) given constraints of resources overtime. Each time period is linked through availability of resources and activities as appropriate. In designing MPDLP models decisions ought to be taken on, length of the time horizon, length of intervals within the time horizon, the rate of inter-temporal time preference (interest rate), risk conditions if such is to be considered in decision making (Cembalo, 2002).

Data Collection Methods

The core datum required for this study is the age-yield relationship of date palm varieties cultivated in the Sultanate of Oman. A survey was done to obtain above datum through a purposive sample of farms. The sample included farmers from whom above information could be reliably obtained, i.e., farmers with commercial date palm cultivations with more than 2.4 hectares and are elderly farmers who had a memory of age-yield relationship of date palm. The sample size was limited to 34 farmers given the nature of data collected that required extensive time to interview the respondents and the limitation of other resources as finance and personal.

RESULTS AND DISCUSSION

Base Data Used in CEAN and MPDLP Models

The average yield is higher than nationally reported yields of 45 Kg/Palm, since the sample was purposive and represented large commercial farms. Using the data in table 1 a best-fit curve on the age-yield relationship of the khalas date cultivar was estimated (figure 2). Number of palms per hectare was considered as 125 as found by the survey. It was considered that the same cultivar (khalas) is replanted, hence the same age-yield relationship was considered for the potential replanting palms. Sensitivity analysis for an increase and decrease of 25% of the base yield was done. Using the average price of dates over the period 1961 to 2011 in Oman as reported in (FAO statistics) a trend line on price prediction was estimated. Based on the trend line the predicted price of 0.250 OR/Kg was considered as the base price. A decrease and increase of 25% of the base price was considered for sensitivity analysis. Cost of production estimate of 914 OR/hectare for date palm cultivation provided by the Ministry of Agriculture and Fisheries Wealth, Oman was used as base cost. An increase and a decrease of cost by 25% was considered for sensitivity analysis. A 4 % interest rate was in the study based on the average for the period 2002 to 2010 of the interest rate spread of Oman as reported by the World Bank. Sensitivity analysis was done for 1% and 10% interest rates.

Results from CEAN Model Analysis

It is estimated that on base conditions the optimal age to replant is 52 years. The optimal age of replanting is sensitive only to the interest rate. A decrease in the interest rate (1%) shortens optimal replanting age to 49 years and an increase interest rate (10%) lengthens the optimal replanting age to 56 year.

Results from MPDLP Model Analysis

The MPDLP model was solved for base data and then sensitivity analysis was done on price, yield and interest rate changes. The MPDLP model was solved for the same scenarios solved by the CEAN model. The MPDLP model however produces discrete results on (age as a range of 5 years) whilst the CEAN model is continuous on age. Considering results of both models and results of sensitivity analysis on price, cost, interest and yield changes it could be concluded that date palms should be replanted at about 50 years.

Replanting Schedule Date Palm Plantations in Oman

Data on age distribution of date palm plantation in Oman is not available. An estimation was made using the (FAO STAT) data on area harvested. The area planted with date palm at a given period increases the area harvested after 5 years as fruiting occurs after 5 years of planting. It is estimated that from the extent of date palm area in year 2000 (35508 Ha) at least 14000 Ha (or about 40%) is older than 40 years. The MPDLP model was used to estimate the replanting schedule (extent to replant by periods) for Oman . The extents in hectares in the second column of the table 5 are the extents that should be replanted in the identified periods of the first column. The model estimated that if replanted optimally it will generate net revenue 2218 Million OR and if not replanted the net revenue will be 1856 Million OR in present value over 60 years and 4% interest. The benefit of replanting over not replanting is 361 Million OR in present value, which is equivalent 16 Million OR when amortized over 60 years and 4% interest. This implies that if a replanting is undertaken as scheduled in table 4 Oman could gain 16 Million OR per year in the future. However this is an upper-bound estimate because the sample considered for this study is a purposive sample of large commercial farms reporting an average yield of 132 Kg/Palm. This yield is 3.5 times higher than the national average date palm yield of 38 Kg/Palm. Also the upper-bound estimate assumes that at present farmers do not replant date palm. However the survey found that 65% of the farmers responded of having replanted (at least one palm) date palm in the past years. Thus this is an upper bound estimate of rate of replanting adopted. Given above reasons the upper bound value was subjected to a sensitivity analysis by considering the national average yield of 38 Kg/Palm and a 65% rate of replanting as being currently adopted. Thus, average benefit from replanting date palm based on the sensitivity analysis is 7 Million OR/Year. The revenue from date palm in year 2011 in Oman was 52.6 Million OR (FAO STAT). Thus replanting of date palm could increase the revenue by more than 13% over the current (2011) revenue from date palm, in the Sultanate of Oman.

The optimal age to replant is where discounted marginal net-revenues (MNR) is equal to the annuity formed from the discounted total flow of net-revenues (ANR) from date palm yields of palms replanted over years. The study estimated the optimal age of replanting date palm as 50-55 years. Both models, CEAN and MPDLP gave consistent estimates. The optimal age to replant date palm was sensitive only to changes in the interest rates. Low interest rates shortened the optimal age of date palm replanting. The study derived the optimal replanting schedule for date palm for the Sultanate of Oman given the current age distribution of date palms. On the average, the benefit of replanting date palm for the Sultanate

of Oman is about 7 million OR/year. The study reveals the significant benefit the Sultanate of Oman could gain by initiating a national program to encourage farmers to replant old unproductive date palms. Government would also have to plan extension activities and allocate resources for such a program. Since replanting, unless well planned at the farm level would incur reductions in the farm cash flow for short periods, cash flow support through financial institutions and/or government subsidies could be considered to encourage farmers. The government could also support farmers through propagation and providing such high yielding cultivars with modern technology such as tissue culture etc. The data used for the study was restrictive in terms of the sample size and the due to the adoption of purposive sampling method. This however was rather inevitable given the nature of data to be collected, particularly the age-yield relationship of date palm. It is recommended that agronomic research be undertaken to establish the age-yield relationship of this vital crop. A larger random sample and a more elaborate data collections process, involving more financial, personnel and time resources could have improved the validity of the study. The analytical methodology adopted in the study was theoretically and operationally robust. Both models namely CEAN and MPDLP models can be used by extension officers in developing date palm replanting schedules and advising farmers. Despite some limitations of the study, the findings of the study suggests the need to adopt a date palm replanting program to improve the date palm sectors' contribution to the society and economy of the Sultanate of Oman.

Acknowledgments

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Figures

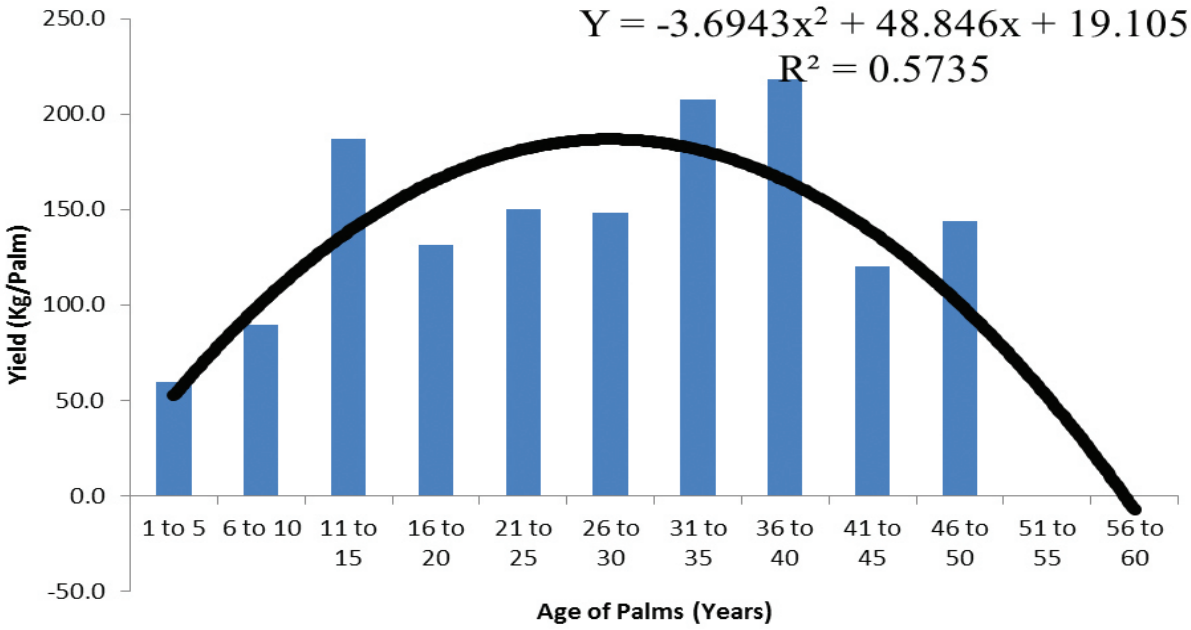

Fig. 1. Age-yield relationship of khalas date cultivar and the estimated function.

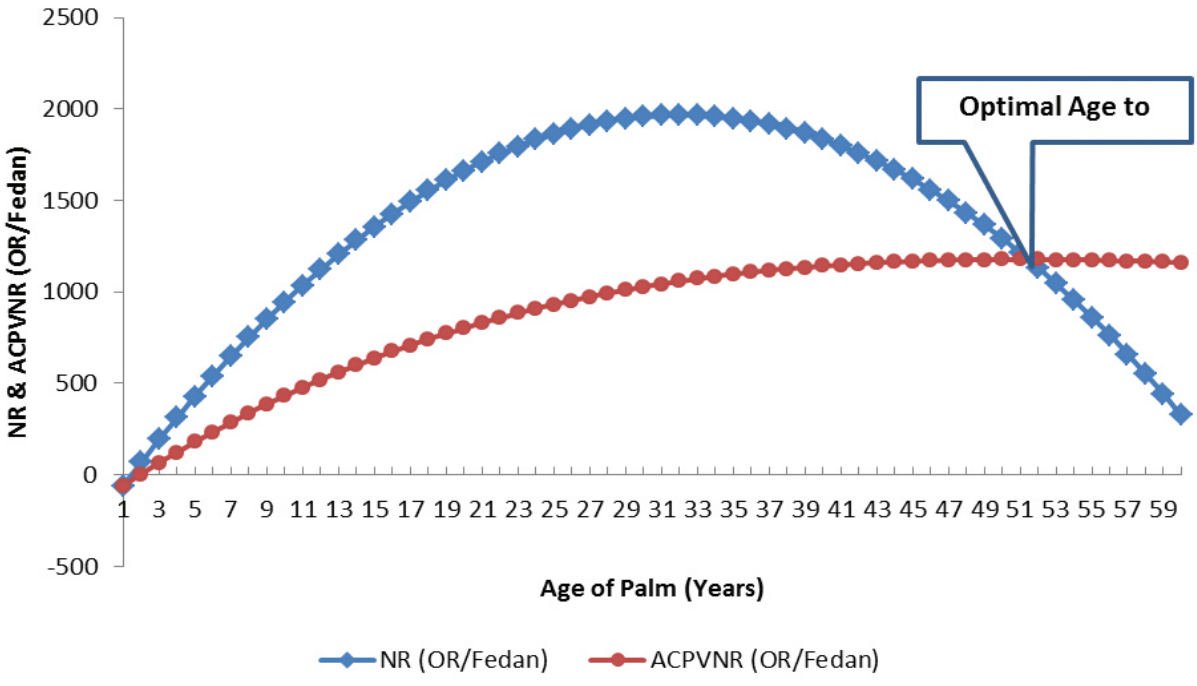

Fig. 2 Results of the base model with age-yield relationship of Khalas cultivar

Tables

Table 1. Age-yield relationship for khalas date cultivar.

Age of Palm (Years)		Yield (Kg/Palm)	
		Survey	Predicted
1 to 5		59.4	62.3
6 to 10		89.5	102.0
11 to 15		187.2	133.7
16 to 20		131.7	157.2
21 to 25		150.0	172.7
26 to 30		148.0	180.0
31 to 35		207.5	179.3
36 to 40		218.0	170.5
41 to 45		120.0	153.6
46 to 50		144.0	128.6
51 to 55		NR	95.6
56 to 60		NR	54.4
Average	145.5	132.5	

Table 2. Results on optimal age of replanting and sensitivity analysis for Khalas cultivar: CEAN Model

Sensitivity Analysis									
Factors	Base Model	Price Change		Cost Change		Interest Change		Change in Yield	
		Increase by 25%	Decrease by 25%	Increase by 25%	Decrease by 25%	Increase	Decrease	Increase by 25%	Decrease by 25%
Age (Year)	60	60	60	60	60	60	60	60	60
Average Yield (Kg/Year/Tree)	132	132	132	132	132	132	132	165	99
Average Yield (Kg/Hectare)	16450	16450	16450	16450	16450	16450	16450	20561	12336
Price (OR/Kg)	0.25	0.31	0.19	0.25	0.25	0.25	0.25	0.25	0.25
Average Gross Return (OR/ Hectare)	4111	5261	3225	4111	4111	4111	4111	5141	3084
Average Cost (OR/Hectare)	914	914	914	1142	686	914	914	914	914
Average Net Return (OR/ Hectare)	3197	4186	2210	2969	3427	3197	3197	4226	2170

Sensitivity Analysis									
Factors	Base Model	Price Change		Cost Change		Interest Change		Change in Yield	
Interest	0.04	0.04	0.04	0.04	0.04	0.10	0.01	0.04	0.04
Year of Replanting	52	52	52	52	52	56	49	52	52

Table 3. Results on optimal period of replanting and sensitivity analysis on age yield relationship of Khalas date palm: MPDLP Model.

Sensitivity Analysis									
Factors	Base Model	Price		Cost		Interest		Yield	
		Increase by 25%	Decrease by 25%	Increase by 25%	Decrease by 25%	Increase to 10%	Decrease to 1%	Increase by 25%	Decrease by 25%
Age (Year)	60	60	60	60	60	60	60	60	60
Average Yield (Kg/Year/Tree)	132	132	132	132	132	132	132	165	99
Average Yield (Kg/Hectare)	16449.6	16449.6	16449.6	16449.6	16449.6	16449.6	16449.6	20560.8	12336
Price (OR/Kg)	0.25	0.31	0.19	0.25	0.25	0.25	0.25	0.25	0.25
Average Gross Return (OR/ Hectare)	4111.2	5260.8	3225.6	4111.2	4111.2	4111.2	4111.2	5140.8	3084
Average Cost (OR/Hectare)	914.4	914.4	914.4	1142.4	686.4	914.4	914.4	914.4	914.4
Average Net Return (OR/ Hectare)	3196.8	4185.6	2210.4	2968.8	3427.2	3196.8	3196.8	4226.4	2169.6
Interest	0.04	0.04	0.04	0.04	0.04	0.10	0.01	0.04	0.04
Age of Replanting (Years)	55	55	55	55	55	55	50	55	55

Table 4. Date palm replanting schedule for the Sultanate of Oman.

Year/Period	Extent (Ha) to be Replanted
2010-2015	13000
2016-2020	0
2021-2025	1000
2026-2030	6100

Year/Period	Extent (Ha) to be Replanted
2031-2035	4900
2036-2040	4000
2041-2045	6508
2046-2050	0
2051-2055	0
2056-2060	0
2061-2065	0
2066-2070	13000

Table 5. Estimated benefits of replanting date palm plantation in the Sultanate of Oman.

Scenario	Present Value (Million OR)	Amortized Value (Million OR)
Without replanting	1856	82
With replanting	2218	98
Benefit of replanting	361	16

Table 6. Sensitivity analysis on benefits of replanting date palm in Oman.

Variables		
Yield (Kg/Palm)	% Current Replanting Rate	Benefit (Million OR/Year)
132.0	0.0	16.0
38.0	0.0	4.6
132.0	60.0	5.6
38.0	60.0	1.6
Average		7.0

Challenges of Algeria exports dates; in light of the current competition

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ABSTRACT

Algeria, for its workforce of more than 18 million palm trees and 952 varieties, occupies an important place among countries producing and exporting dates in the world, even more, it ranks first in terms of quality, through the famous variety Deglet Nour. Despite the policies of the country since independence to develop the agriculture, which has paid great importance to the promotion of export, but several studies have shown that exports of Algerian dates are several obstacles and did not reach the fixed objectives, despite the comparative advantage available to it in the country. Its position in the list of countries that export this product retreated to eighth place in recent years. And exported quantities represent only 3% of national production in 2012 after it was almost 14% after independence. The bulk of these exports is limited in most cases of the variety Deglet Nour and ignored the other species. As well as the lack of diversity and global markets and focus on traditional markets, especially the French more than 80%. While other destinations for exports are much lower, they are cyclical and irregular. We will try, through this intervention to highlight the reality of Algerian exports of dates, as well as major impediments to its development, and the deterioration of its competitiveness, especially in the current changing world market dates: Expansion and increased competition. This weakness in exports dates in Algeria is mainly due to the combination of a series of technical constraints, socio-economic, natural, agricultural and administrative. One of the main causes is the lack of competitiveness in the international market and the instability of its position in key markets. and dysfunction of the chain date upstream and downstream. (Poor performance technical and economic).

Key word: Policies, promotion of export, dates, obstacles, comparative advantage, competitiveness, decline.

INTRODUCTION

The socio- economic and environmental importance of the phoeniciculture is far from negligible in the world (Dubost, D. 1990). It is regarded as the central pivot around which life revolves in the Saharan regions. In Algeria, the crop occupies a top position in the Saharan agriculture, mainly through its economic interest throughout, the settlement of more than 2.2 million inhabitants of the population Saharan areas, the employment market that it provides, the product it offers on the national and international market and by currency generated. (Benziouche, S. 2008)

Algeria, with more than 18 million palm trees and 952 varieties, occupies an important place among the countries producing and exporting dates, more, it ranks first in terms of quality, thanks to the famous variety Deglet Nour.. Algeria is currently representing 6.75% of world production of dates and it ranks sixth, it also carries 3.20% of world exports and also ranks eighth. Its exports contribute 48.25% of total export value of agricultural products .

However, these data do not reflect the true image of the dates sector in Algeria, many studies (Moulay Lakhdar, A. 1995. Benziouche, S.2000) showed that in this sector there are many difficulties in its operation at all levels (production and marketing) and fails to achieve its objectives since independence to date, although various policies implemented in this area. His place in the list of countries producing and exporting is in continuing decline. In parallel, the degree of integration of Algeria into the dates world market, has

undergone considerable change downward until 4% in the last decade while he was over 25% in the sixties.

Other hand, if the production dates of Algeria is 3 times the production Tunisian, However exports of this country are higher than that of Algeria for four times. Although the agricultural policies implemented by the State to promote the sector since independence, but it has not achieved the objectives set

Through this communication, we try to identify the level of exports of dates, to analyze the dynamics of evolution and highlight the obstacles that hinder the development of exports of this fruit. So our problem is:

What are the constraints that prevent the growth of exports of dates in Algeria? ;

2. MATERIAL AND METHOD

In this work we have based on statistics collected from the Algerian Customs and FAO and field surveys at certain exporters of dates and of certain institutions with connections to the subject of exports. Thus, the steps of our methodology are:

Initially, we diagnose the situation of export of dates in Algeria, through an analysis of trends in quantity and value, and their structures by variety and destinations, and this during an analysis period that stretches from 1990 until 2011 to determine the general trend of exports. For this, we calculate regression functions after making tables and graphs of different types. Subsequently, we analyze the results of the latter with the use of survey results. This type of analysis allows us to illustrate the degree of disruption of exports and the causes of this situation.

3. RESULTS AND DISCUSSION

3.1. Analytical study of exports of dates in Algeria:

3.1.1. Position of Algerian dates in the international market.

Algeria is ranked seventh with 3.20% of world exports of dates on average during the period (1990-2011) estimated at 621. 52 thousand t. This share is still small compared to the United Arab Emirates,, Pakistan, Iran Tunisia, Saudi Arabia, who seized 28.11%, 14.38%, 13%, / and 9.96%, respectively (Table 1).

Examination of data (Table 2) revealed that the ranking of the largest exporters of dates depending on the value has changed completely. Indeed Tunisia took the lead from the list of exporting countries with an amount 60.14 million of U.S. $, or 26.41% of the total value of world exports during the period (483.67 million U.S. $). This reflects the high quality of its dates. Second is Iran with $ 30.97 million or 13.60% while third place is occupied by Algeria with an average value of 28.48 million U.S. $ or 12.50%. Pakistan, Saudi Arabia and France occupy the next three spots with an average export value of 22.88, 21.76, and 20.41 million dollars or 10.05%, 9.55% and 8.96% respectively.

3.1.2. Development of exports of dates in Algeria

Despite the increase in production, development of exports of dates from independence to date shows that Algeria exported only 12 093,67 T in average during the period 1990-2011 (Benziouche, S 2012), with 28143 t in 2011, and a minimum of 3763 t in 1994 (Figure 1). with Average annual rate of evolution does not exceed 36.6%. This evolution is characterized by: the sequential decline and volatility and instability in the development.

Indeed 89% of variation explained by internal factors, such as weakness in the sector and the poor quality of dates caused by the misconduct of palm, but also by climatic factors. And secondly, by external factors, including competitive pressure from Tunisia and the saturation of markets, which weigh all their weight. And thirdly by the constraints faced by exporters of dates and non-effectiveness of policies.

In parallel, (Figure 2) reveals that the average value of exports of dates of Algeria is 26 million U.S. $ in the period 1990-2011, and that this value varies between a minimum of 10.44 million U.S. $ in 2001, and a maximum of 79.12 million U.S. $ recorded in 1995. Moreover, the study confirms the general trend of increase of this value with an annual growth rate of 566.7 U.S. $ thousand, or an annual growth rate of 3.70%. These changes are attributed to 82% by the average price increase for export.

3.1.3. Evolution of the average price for export of Algerian dates.

The average price of exports experienced a general trend upward with a statistically significant annual growth, estimated at 46.72US $ / t. However, despite an increase of 341US $ / t in 1964 to 1744.60 U.S. $ / t in 2011, it remains below the average export price of the dates of some competing countries such as Tunisia (over 2300 U.S. $ / t in 2011). However, it remains above the average price of Iran and Saudi Arabia where it does not exceed 284 U.S. $ / t and 671 U.S. $ / t respectively. This is explained by 48% by the quality and variety type of dates exported as Deglet Nour while 52% for those changes back to other factors such as speculation in the markets. This helps to explain the decline in external demand.

3.1.4. Evolution of the rate of integration of the Algerian date.

This integration level (expressed by the ratio between the quantities exported dates and production of this fruit in the same period), know a irregular trend during the study period 1964-2011 (Figure 3). Indeed, after a slight increase during the first 3 years, which reduces from 15.75% in 1964 to 24.32% in 1966, it was subsequently submitted to a downward trend that reduces the level of 2.22% in 2011, it recorded its maximum of 24.32% in 1966, and the minimum in 1982 of 0.5%. While the average ratio during the entire period is estimated at 4.62%.

3.1.5. Structure of dates exports by variety and destination.

The analysis of the structure (Figure 4) exports of dates by variety (according to the classification of Customs) shows that Algeria's main exports the Deglet Nour and other small quantities of fresh dates as Tafzuine. this this variety, although slightly declining, remains predominant with 86% of the average quantity of dates exported between 1990 and pre 2011 and over 94% in value (Benziouche, S. 2012) . Regarding the soft dates, two groups dominate: the Ghars and date paste that 12% of total export volume during the period. The remainder consists of dry dates and the like (Degla-Beida, Mech-Degla) which represent only a very small proportion of 2% in export volume.

The Most of these exports is mainly for sale to the European Union, which represents the traditional client the largest and most stable of Algeria with 94% of Algerian exports of dates in value and 95% of the quantities exported betwen 2000-2011. In contrast, the French market remains the main partner with an import of 77% in quantity and 80% and the value of dates exported by Algeria during the period 2000-2011. This is the result of lack of efforts to diversify exports to other European markets.

3.2. Analytical study of problems of Algerian exports dates.

3.2.1. Constraints to production.

One of the main causes for weak exports dates in Algeria and the unstable position of the dates in major markets is primarily problems associated with the production of palm that are characterized by low yields of the palms and productivity in both quantity and quality due to the advanced age of the plantations because much of the palm trees over the age limit of production (80 years). This situation is aggravated by the low or even lack of renovation and expansion of new which would be aimed at both reducing the excessive density of trees and replacement palms. (Benziouche, S. 2005).

Bad, or even the lack of application of crop management in the conduct of the date palm cultivation. (Benziouche, S, F. Chehat. 2010). Indeed, it is clear that the orchards were abandoned because the maintenance of palms are rarely performed work such as soil, compliance with technical standards, cleaning orchards, which is regarded as a means to fight against pests dates, causing damage of 20 to 30% of harvest.

The low level of mechanization and the phenomenon of abandonment of oasis worsened since the advent of oil and especially with the growing number of economic sectors in the country. Work in the oasis appears increasingly burdensome for the local workforce in general, what makes that available to require compensation reaching high levels for some operations.

In most oases, the drainage problem has not ceased to exist. Increased irrigation water has further promoted the rise of the level of excess water that must be evacuated. In this sense, the inadequacy or lack of sanitation in the oasis is one of the main constraints to production-palm. This constraint has resulted in reduced production and a depreciation of the quality of date. (Bouamar, B. 2009).

The persistent water shortage in some oases generates low levels of satisfaction with irrigation as the irrigation rate not exceed 0.41l/s/ha in the oases of the region, a flow of 24,24 l / s and around the day of water exceeds 14 days. Among the constraints that are causing the low irrigation water, shortage and under-utilization of resources.(Benziouche, S and F, Cheriet. 2012)

The degree of attack of pests and diseases deferens is very high in the oasis. These pests and diseases cause significant impairment rather on production on the year and date palms region (Bouamar, B. 2009). Accentuated by the low fertilization and amendment en raison d'un handicap financier. tellement que certains phoeniciculteurs abandonné les traitements chimiques.

The low skills and lack of knowledge of the application of technical crops, accentue le besoin de sensibilisation et de formation, which until now has not resolved the technical problems of production and did not improve know-how.

3.2.2. Constraints related to marketing.

Regarding the constraints links marketing phase the most ultimate expression of income, are numerous including:

The lack of promotion of dates, especially for categories of so-called common varieties that remain unknown, severely limits the absorption capacity of the product at level nationally and internationally.

The lack of control over the quality aspect at farms and packing units. Secondly, packaging dates packing units that do not meet the requirements of the different distribution channels, in addition to high costs operations of harvesting, sorting and transportation that affect the price of dates that are no longer competitive on the international market..

The low flow of Algerian dates on the world market following the difficulties to retain existing markets and penetrating new markets. This situation resulted from the bad image on the dates given by some Algerian operators and secondly, due to lack of credit given to exporters on the world market.

The image dates strongly degraded by Algerian exports anarchic performed by non professionals. and non-proper application of laws enacted within its organization.

On the other hand, exports are governed by any rules of organization of the profession, especially when it comes to selection criteria specific to the export or distribution of export licenses. These exporters because of bureaucratic and administrative barriers at institutions involved in the export process (banks and customs and ports) and the lack of offensive spirit and consciousness among some exporters, this affects consequently on the credibility of Algerian exports dates.

The absence of a bold policy of integration and coordination that brings together exporters and other stakeholders in the profession and even some state institutions (embassies) hinders competitiveness, strengthening and consolidation of bargaining power scattered vis-à-vis the European importers and cope with foreign competition and penetrate new untapped markets in other regions.

The situation is also explained by:

- The limitation of Algerian exports to neighboring markets only traditional, effortlessly wise use of American markets, Asian and other markets promoters date.
- Exporters face major difficulties in the reservation and the landing of their containers following the anarchy that characterized the Algerian ports and insufficient of means(handling, storage and security).
- Lack of advertising culture in the minds of some officials of units of export.
- The mismatch between the packaging used by our exporters and international standards for packaging, is a major disability to compete with neighboring countries.
  The lack of rigor in applying the measures of quality of dates in the control and packaging establishments.
- The lack of programs to control pest of dates atØ all levels and non-rigorous application of existing laws dealing with this issue and at this level are many.

CONCLUSION

The main conclusion of this work, using data from surveys conducted on land at all levels of the division dates in Algeria is that exports of dates in Algeria are still weak and far from the objectives and effects and standards expected. This deteriorating situation resulting primarily from a series of constraints organizational, technical, socioeconomic, natural, agricultural and administrative. Despite the efforts and measures put in place to strengthen and promote exports of dates in the global market, particularly with the emergence of new competitors on the European market, but it seems that these policies have not yielded the required level and did not yield acceptable results,

Given this situation it is necessary to restructure the structures and institutions for foreign trade, and create other structures specifically to promote exports of dates. In addition, this sector will be integrated into the market economy, will not play this role if it has the adaptability and competitiveness. This will not be possible without the removal of various barriers at all levels.

And although the results are recorded, the dates division in Algeria is very efficient economically, particularly with the substantial income that is distributed to families in rural oasis, the reduction of rural depopulation and agricultural offers temporary employment created by all the actors in this sector, not forget the foreign exchange earnings generated by the export of this product.

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Tables:

Table 1. Ranking of countries exporting quantity of dates (1990-2011). *Source: (Algerian Customs, 2012, FAO, 2012.)*

Country	Average 1000T	Structure in%	Standings
E.A.U	96,41	25,68	1
Irn	92,67	24,68	2
Pakistan	57,05	15,19	3
Tunisia	27,74	7,39	4
Saudi Arabia	25,20	6,71	5
Iraq	15,17	4,04	6
Algeria	12,29	3,27	7
France	7,63	2,03	8
Oman	6,27	1,67	9
Israel	5,02	1,34	10
USA	4,77	1,27	11
China	3,64	0,97	12
Egypt	3,30	0,88	13
Other countries	18,34	4,88	14
Total world	**375,48**	**100,00**	

Table. 2. Classification of countries exporting dates by value during the period 1990-2011. *Source: (Algerian Customs, 2012, FAO, 2012.)*

Country	Structure in%	Average millions $	Standings
Tunisia	26,41	60,14	1
Iran	13,60	30,97	2
Algeria	12,50	28,48	3
Pakistan	10,05	22,88	4
Saudi Arabia	9,55	21,76	5
France	8,96	20,41	6
Israel	7,87	17,92	7

Country	Structure in%	Average millions $	Standings
E.A.U	7,34	16,73	8
USA	5,84	13,30	9
China	2,13	4,85	10
Oman	1,33	3,03	11
Iraq	1,28	2,92	12
Other countries	4,32	9,83	13
Total world	**100,00**	**227,74**	

Figures:

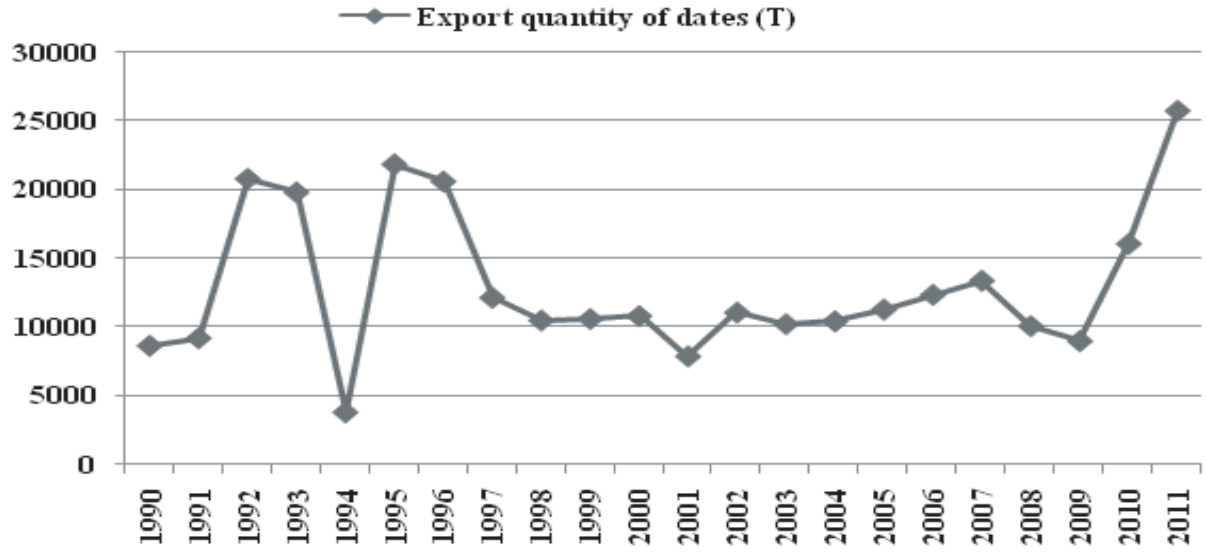

Fig. 1. Evolution of quantities of dates exported during the period 1990-2011 (T).

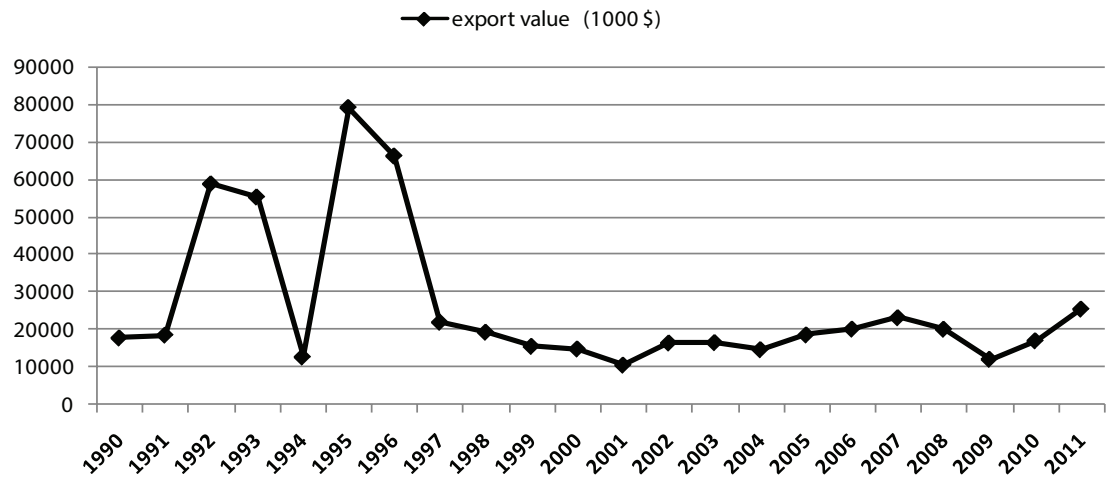

Fig. 2. Evolution of Algerian exports of dates in value between 1990-2011 ($ 1000)

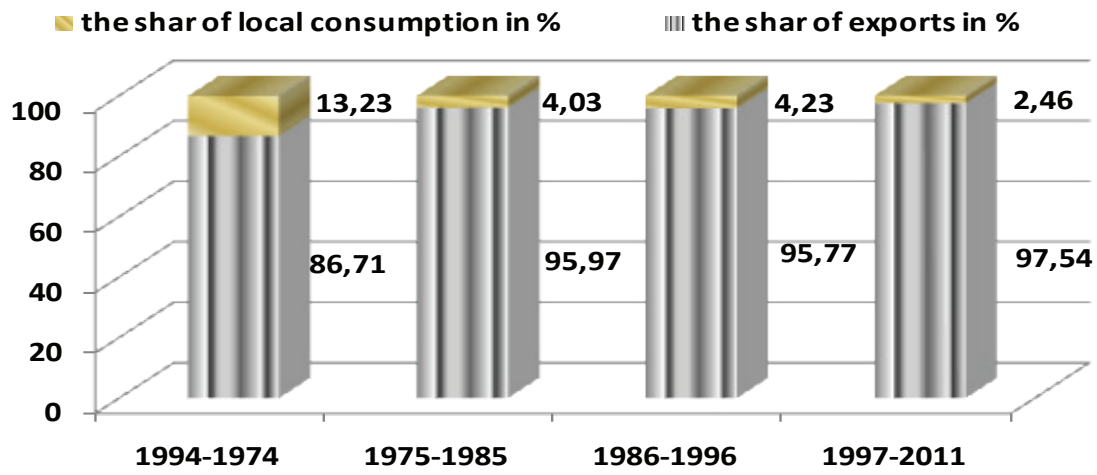

Fig. 3. Evolution of the integration rate of Algerian dates during 1964-2011 (%)

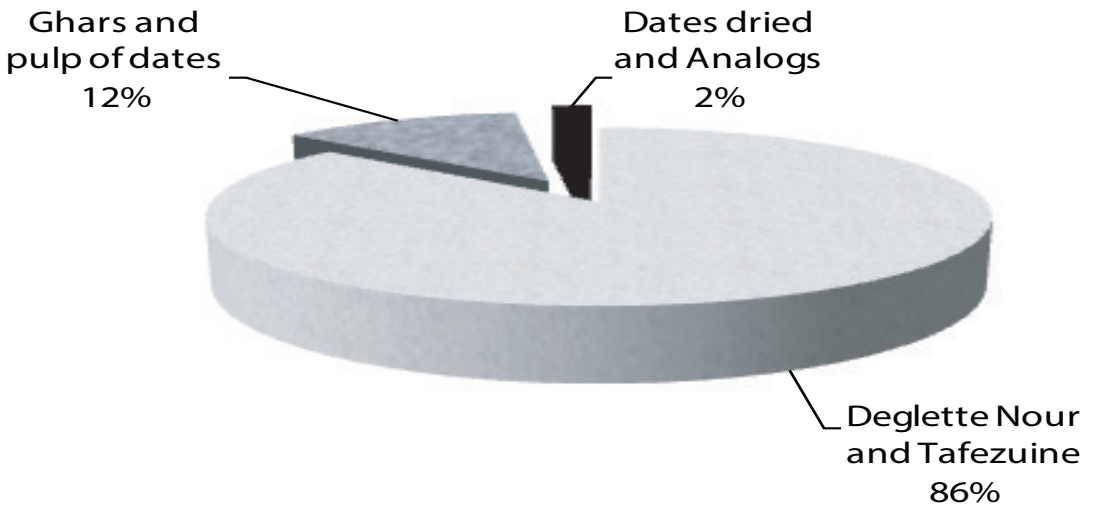

Fig. 4. Structure of exports by variety of dates in 1990/2011

Assessment of nutritional status in date palm (*Phoenix dactylifera*) orchards of cv. piarom through deviation from optimum percentage (DOP) method

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ABSTRACT

Many studies have been carried out in order to determine an accurate and proper method for assessment of nutritional status in plants. Current methods include both soil and plant tissues analysis. Plant analysis is a useful technique for evaluating the nutritional status of plants and can be used accompanied with soil test results in balanced fertilization programs, evaluating the use efficiency of nutrients by the plant. "Deviation from Optimum Percentage" (DOP) is a new and simplemethod compared to the oldermethod named "Diagnosis and Recommendation Integrated System" (DRIS) used forthe interpretation of foliar analysis. Meanwhile, calculation ofindividual nutrient index is much easier with DOP. This study was accomplished to estimatethe DOP of date palm cv. Piarom throughout 2012 growing season across private orchards in Hormozgan province, Iran. Foliar samples were collected in October from 39 date orchards. Average yield and concentration of N, P, K, Ca, Mg, Fe, Cu, Zn, Mn, B, Cl and Na in leaves were determined.The DOP index was calculated for each measured element using the following equation: DOP = $[(C \times 100)/C_R]$ - 100, where C is an element concentration in the foliar dry matter, and C_R is the optimal concentration for the same conditions.Results showed that there was an imbalanced nutritional status in the orchards. The calculated average DOP indices in low-yield date palm orchard of cv. Piarom determined the required nutrients orderas: P > B > Zn > Ca > Fe > Mg > Mn > N > Cl > Na > Cu > K. Therefore, we strongly recommend the application of P and micronutrients fertilizers in date (cv. Piarom) orchards of Haji Abad area in Hormozgan provice.

Keywords: Leaf, Nutrient Balance, Nutrient Concentration, Optimal Concentration, Fertilizers.

INTRODUCTION

Date palm is a major agricultural crop in the Near East and North Africa, and it has historically beenconnected with sustaining human life in many of the hot and barren parts of the old world and has becomean integral part of the culture and tradition of the people of these regions (El-Juhany, 2010). Date palm is one of the most important trees in calcareous soils in southern region of Iran."Piarom" date palm has a high quality and a good market in the world (Saleh, 2009).Although plant mineral nutrition and optimization of mineral supply has been the subject of numerous studies for decades since the 19th century, today there is still controversy about the methods for diagnostics

and fertilizer management designed to obtain optimal plant productivity and sustainability in agriculture (Osvalde, 2011).The search for an effective method to determine plant nutritional status has been the target of many of thestudies in the area of plant nutrition. Current methods include both soil and tissues analysis (MourãoFilho, 2004).Plant analysis technique is beneficial for evaluating the nutritional status of plants. This method can be used accompanied with soil test results in balanced fertilization programs to evaluate the use efficiency of nutrients in the plant. In addition to considering the timing samples, a standard technique of sampling and analysis, the plant analysis method efficiency is depended on the interpretation of the data of the analysis (Montanes *et al.,* 1993).Different ways of interpreting theresultsofplant analysis arecriticalconcentration, sufficiency level, and DRIS (Tisdale *et al.,* 1990; MourãoFilho, 2004; Osvalde, 2011). Criticalconcentrationisa range ofnutrientconcentrations thatin the lowerborder,plant yield starts to be declined, compared toplants with highernutrient concentrations.In other words, 90 to 95% of maximum yield could be observedin this concentration level.InDRISmethod,an index is calculatedusing the ratio ofnutrientsfor eachelement, which can be found as quantity relativebalanceof nutrientsandnutrient requirements. Unlike as deficiency approachand the criticalconcentration, diagnosis at any stage of plant growths is possible in DRISmethod.However, theabsence ofreliablereferencenorm for many of plant is a practical problem for using this method.Furthermore, DRIS uses nutrient ratios instead of absolute and/or individual nutrient concentrations for interpretation of tissue analysis (MourãoFilho, 2004). In contrast, a simple method was developed, named as Deviation from the Optimum Percentage (DOP).This method is an improvement of the critical levelmethod. It evaluates individual nutrient concentration in relation to the optimum value (Osvalde, 2011). In this method, an index (value) is calculated for eachnutrient. This value can be positive, negative or zero, indicating high, deficiency or suitable concentration of nutrient in the plant, respectively. Some researchers employed DOP method to evaluate the nutritional status of plants (Goudarzi, 2005 in vineyards; Monge at al., 1995 in peach; Samadi and Majidi, 2011 in grape).

The objective of thisstudy was toevaluate nutrientstatus ofdate palmcv. “Piarom” in Hormozgan province using DOP and determine the nutritionalstatus of the plant and the arrangement of the elements as a function of the degree of deficiency.

MATERIALS AND METHODS

The experiment was conducted at private orchards of Haji Abad area, Homozgan province of Iran on date palm cultivar ‘Piarom’ in 2012 growing seasons.Leaf samples were collected in October 2012 from 39 orchards (5 trees in each orchard were sampled, and then were combined). Samples were washed with tap water,HCl, distilled water and dried in an oven at 70° C for 48 hrs. Dried samples were ground in a stainless steel mill with 0.5 mm sieve,and then digested.Concentrations of N (using Micro- kjeldahl method), P and B (via colorimetry), K and Na (through flame-photometery),Ca, Mg, Fe, Cu, Zn and Mn (using atomic absorption spectrometery)were determined in leaves. Averageyieldwas also determined. In the next step, the orchards were divided into two groups, one group with high yield and the other one showing low yield. Boundaryseparatingthetwo communitieswas50kg pertree. The averageconcentration ofnutrientelementsin samplesfromorchardswithahigh yield (more than50kg pertree) was selected as standardizedandoptimizednutrient concentration. In order to determine thedeviationfromthe optimum percentage (DOP)for eachelementin orchards with low yield (less than50kg pertree), DOP index was calculated. This index evaluates the nutrient concentration in relation to the optimum value by the expression: DOP= [(C×100/ C_R)-100], where C is the element concentration in the leaf dry matter sample and C_R is the optimal concentration for the same conditions (Mello Prado and Caione, 2012).

RESULTS AND DISCUSSION

Table 1shows Mean, Coefficient of Variation and Standard Deviation of nutrient concentrations in leaves belonging to the treeswith high yield. The nutrient concentration in these trees was used as standard nutrient concentration for calculatingthe indices of deviationfromthe optimum percentage (Montanesetal, 1993).

Table 2 shows indices of DOP and required nutrient order for date palm (cv. Piarom) orchards with low yield. The indicesare positive, 0 and negative. Zero value for a nutrient indicates the balanced (optimum) status for that nutrient inthe orchard. Positive and negative value shows excess and deficiency of a nutrient concentration in leavesof trees in an orchard, respectively.

The average of DOP indices in date (cv. “Piarom”) orchards with low yield were calculated and required nutrients order was determined as follows:

P> B > Zn > Ca > Fe > Mg > Mn > N > Cl > Na > Cu > K

Results showed that there is an imbalanced nutritional status in the orchards. The required nutrients order indicates that P deficiency is higher than other nutrients. It may be due to the calcareous soils in this area, causingphosphorus to be fixed as apatite compounds. B and Zn deficiencies were placed in next orders. This seems reasonable, because application of micronutrients is not common in date (cv. piarom)

orchards. According to the results, almost 90% of the low-yielding orchards were faced with micronutrients deficiency. Other researchers also reported that use of nitrogen and phosphorous (Saleh, 2009) and Fe (Saleh, 2008) fertilizers resulted in increasing date (cv. "Piarom") yield and improved quality of date fruit. Therefore, we strongly recommend the applicationof P and micronutrient fertilizers in date (cv. "Piarom") orchards of Haji Abad area in Hormozgan provice.

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Tables

Table 1: Mean, Coefficient of Variation and Standard Deviation of nutrient concentrations in tree leaves with high yield

Elements	Mean	Coefficient of Variation	Standard Deviation
N (%)	1.167	9.22	0.11
P (%)	0.116	131.38	0.15
K (%)	0.896	37.25	0.33
Ca (%)	0.580	30.19	0.18
Mg (%)	0.299	15.72	0.05
Na (%)	0.017	34.90	0.01
Cl (%)	0.890	15.90	o.14
Zn (mg kg $^{-1}$)	10.211	58.73	6.00
Mn (mg kg $^{-1}$)	61.655	51.67	31.86
Fe (mg kg $^{-1}$)	196.401	18.46	36.25
Cu (mg kg $^{-1}$)	4.972	31.95	1.59
B (mg kg $^{-1}$)	125.459	42.45	53.25

Table 2: Indices of deviation from the optimum percentage for the orchards with low yield

Orchard No.	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
1	-4.60	-25.28	47.37	-22.41	-6.37	3.61	-31.10	-33.21	-15.53	76.07
2	-0.82	-37.30	84.21	-39.66	-13.06	-40.12	-15.71	-48.59	-5.08	-21.01
3	14.78	-29.57	22.81	-10.34	3.66	-22.30	-8.98	-48.68	-5.08	-2.36
4	11.35	-23.56	19.46	-32.76	-3.03	-18.84	27.60	-43.49	37.16	113.77
5	9.20	-28.71	-44.18	12.07	0.32	49.03	-34.49	23.29	5.38	30.88
6	-0.57	-5.52	80.86	-36.21	-33.12	-33.17	-37.85	-7.46	26.70	-63.57
7	9.80	-5.52	-12.92	10.34	-3.03	14.15	-34.44	-7.46	-5.08	14.94
8	-5.88	-30.43	63.00	-3.45	-6.37	-6.52	16.55	23.29	47.62	-67.39
9	0.72	-48.47	32.85	-12.07	-6.37	-33.17	26.87	-29.00	-47.31	-56.80
10	9.80	-12.39	-29.67	27.59	10.35	11.61	0.38	18.10	26.50	-3.00
11	7.32	-39.88	64.11	-34.48	-13.06	-36.12	37.94	2.83	5.58	-74.75
12	2.60	-29.57	25.04	-27.59	-6.37	-2.97	29.43	-53.78	-26.19	22.43
13	-5.20	-36.44	50.72	-43.10	-23.09	-29.70	-19.96	-53.78	5.58	-51.51
14	-20.54	-28.71	-20.73	13.79	-9.71	27.65	0.38	28.39	47.62	-69.95
15	-8.37	-34.72	-60.93	12.07	17.04	3.18	10.70	-69.15	16.04	-44.13
16	15.46	-15.83	-14.04	24.14	3.66	10.54	-33.65	-17.84	-5.08	-82.65
17	10.75	58.90	182.46	-13.79	-16.40	-24.89	-2.08	-17.74	5.58	0.59
18	4.49	-26.13	66.35	-12.07	-9.71	-0.66	-23.45	13.01	121.42	-72.26
Mean	**2.79**	**-22.17**	**30.93**	**-10.44**	**-6.37**	**-7.15**	**-5.10**	**-17.85**	**13.10**	**-19.48**

Continue from Table 2

Orchard No.	Cl	Na	Requirement order	Yield (kg/tree)
1	14.61	74.00	Zn > Mn > P > Ca > Cu > Mg > N > Fe > Cl > K > Na > B	44
2	20.22	-16.00	Fe > Zn > Ca > P > B > Na > Mn > Mg > Cu > N > Cl > K	34
3	13.48	32.00	Zn > P > Fe > Ca > Mn > Cu > B> Mg > Cl > N > K > Na	22.6
4	3.37	26.00	Zn > Ca > P > Fe > Mg > Cl > N > K > Na > Mn > Cu > B	27
5	-25.84	2.00	K > Mn > P > Cl > Mg > Na > Cu > N > Ca > Zn > B > Fe	34
6	-19.10	2.00	B > Ca > Mn > Fe > Mg > Cl > Zn > P > N > Na > Cu > K	33
7	-12.36	-22.00	Mn > Na > K > Cl > Zn > P > Cu > Mg > N > Ca > Fe > B	36
8	20.22	44.00	B > P > Fe > Mg > N > Ca > Mn > Cl > Zn > Na > Cu > K	26
9	23.60	-4.00	B > P > Cu > Fe > Zn > Ca > Mg > Na > N > Cl > Mn > K	46
10	-12.36	2.00	K > P > Cl > B > Mn > Na > N > Mg > Fe > Zn > Cu > Ca	31.4
11	16.85	-16.00	B > P > Fe > Ca > Na > Mg > Zn > Cu > N > Cl > Mn > K	43
12	24.72	2.00	Zn > P > Ca > Cu > Mg > Fe > Na > N > B > Cl > K > Mn	31
13	14.61	2.00	Zn > B > Ca > P > Fe > Mg > Mn > N > Na > Cu > Cl > K	18.6
14	-14.61	26.00	B > P > K >N > Cl > Mg > Mn > Ca > Na > Fe > Zn > Cu	14
15	-22.47	-28.00	Zn > K > B > P > Na > Cl > N > Fe > Mn > Ca > Cu > Mg	34
16	-11.24	32.00	B > Mn > Zn > P > K > Cl > Cu > Mg > Fe > N > Ca > Na	26
17	4.49	-16.00	Fe > Zn > Mg > Na > Ca > Mn > B > Cl > Cu > N > P > K	29
18	24.72	62.00	B > P > Mn > Ca > Mg > Fe > N > Zn > Cl > Na > K > Cu	34
Mean	3.50	11.3	P > B > Zn > Ca > Fe > Mg > Mn > N > Cl > Na > Cu > K	

Evaluation the efficiency of spinetoram 12SC against dubas bug on the date palm *Ommatissus* binotatus lybicus (Homoptera : tropiduchidae)

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ABSTRACT

Dubas bug *Ommatissus* binotatus lybicus was spread in the most date palm orchards but the infection severity was different from region to another depending on the services operation, closeness or faraway the river and little or dense tree . The highly infection caused weakness and deterioration the date palm and another fruit trees which were planting under date palm because of honeydew and collection of dust and growth of rot fungi, then led the leaf became dry and dead in Iraq . Chemical control still use against Dubas bug . inorder to select active insecticides and safety or less injury to the environment, this study aimed to evaluate new insecticides (spinetoram 12SC) against Dubas bug . The result showed decline in the numbers of this insect, which reached 0.60,0.93,0.73 and 0.46 insect/leaflet after 10 day of spraying for spinetoram (0.50, 0.75 and 1.0 ml/L) and Bifenthrin (2.0 ml/ L) respectively comparing with 9.6 insect/ leaflet in control treatment (water only). Also the insecticides showed high efficiency of killing, was reached 90.7, 86.9, 88.7 and 89.5 % for spinetoram (0.50, 0.75 and 1.0 ml / L) and Bifenthrin (2.0 ml / L) respectively. The field study revealed that spinetoram had low effect on predators of coccinellidae compare with high effect of Binfenthrin. Belong above results, we can use spinetoram with concentrate 0.50 ml /L because it gave the same efficiency when compare with other concentrates, it was useful to reduce using cost in addition to represent new chemical group which useful to prevent this insect from induced resistance against the action of pesticides beside to the low effect on coccinellidae predators, it was useful to use spinetoram with in IPM programme against Dubas bug.

Key word: spinetoram, *Ommatissus binotatus*.

INTRODUCTION

The date palm *Pheonix dactlifera* had economic important for human life since the seniority and yet. Iraq was the date palm habitats since the seniority in the world(2),had more than 600 variety, almost date palm orchards was falling in small area between 0.25 -2.50 ha.it was accomplished 90% of total area which planting with date palm, but the continuous negligent & little care of palm trees in addition to its infection by many pests led to deteriorate the date palm orchards in Iraq, therefor the no. of date palm trees was reduced from 32 million in 1960 to 16.3 million in 1989 (7). The date palm trees was infected by many pests which caused great injuries, estimated by more than 100 million dollar every year but the no. of pests which attacked date palm trees estimated by 280 species of insects & non insects which attacked date palm in different countries of the world(1). one of them dubas bug *Ommatissus binotatus* which was one of the important

insect, its injuries induced by the nymph & adults which was sucking the plant sap from leaflet & fruits during spring & autumn generation, this led to weakness of infected trees or be died if infection lasted many years without control, also the plants which was planting under date palm was affected by honey dew which secreted by dubas bug, the honey dew led to collect the dust & encourage the growth of fungi on the leaflet & fruits which reduce the bioactivity of date palm trees (6).the dubas bug was spreading in date palm planting regions but the infection severity was different from region to anther region which limited by the level of services operations and nearby or far away from rivers or when the date palm orchards were few or high density of the trees in addition to control measurements or whit out control (6). The first control conducted against this pest, was in 1974 at the Abu-Khaseb-Basrah, then used many insecticides against it (3,4,6,8,9,10&11),the chemical control still use in Iraq against this pest and control measurement used against spring generation only but didn't use against Autumn generation to avoid the residues of insecticides on ripen date fruit & protected the consumers from its injuries (6) .the amount of different insecticides which using against this pest estimated by 400-500 ton/year (9). While The chemical control still use in Iraq, there for must be search about new effective insecticides and safety instead of traditional insecticide which used yearly by state board of plant protection, there for this study aimed to evaluate spineforam 12SC which belong to new chemical group with in IPM program .

MATERIAL & METHOD

This study was conducted in the one orchards of date palm at the Al-awarah region-Al-hussainia /Karbala, south of Baghdad –Iraq.it was selected no. of date palm trees at the same ageing &variety (zahdi), the treatment was distributed on these trees which represented by the insecticides, spinetoram 12 sc with concentrate 0.50, 0.75 and 1.0 ml/ L . in addition to control treatment(water only), (3) replicate for every treatment, using RCD design. The spraying operation conducted during the end third week of May by using spraying system, capacity 100 L, manufacture in Turkey, sampling was taking randomly by cutting (5) leaflet /frond and put it in the plastic sac(black color), 15leaflet / treatment. The no. of insect was calculated in the leaflet & in the sac. the result was analyzed statically, the percentage efficacy was calculated by using Henderson & Tilton equation (17). The following description of using pesticides:

1. Spinetoram (Radiiant SC 12 %) – it was new generation of pesticides belong to Spinosyns group .produced by aerobic fermentation for soil bacteria *Saccharopolyspoea spinosa* (Actinomycetes).the spinetoram was mixing of spinosys A(C41H65NO10) and spinosys D (C42H67NO10),LD50 was 5000 mg / kg (orally & dermal for rats) and its chemical structure was 3- ethoxy -5,6-dihydro spinosyn J and the effective material was spinosyn, this insecticides recorded by EPA agency &clacified with in fifth group according to WHO calcification(14, 18).
2. Bifenthrin- it was pythriod compound .LD50 375 mg / kg (orally for rats) . its chemical structure was 2-metyl(1,1- diphenyl) -3-YL} methyl -3 –(2-chloro-3,3,3- triflouro-1- propenyl) -2,2-di- methyl cyclopropane carboxylate.it was recorded in Iraq against dubas bug, was calcified with in second group according to EPA &WHO clacification(12).

RESULTS & DISCUSSION

The study results revealed that the population density of dubas bug was decline after (3) days of spraying operation and go on in reducing, was reached 0.60, 0.93, 0.73 & 0.73 insect / leaflet after (10) days of spraying for insecticides spinetoram with concentrate 0.50,0.75 &1.0 ml/ L and bifnthrin with concentrate 2.0 ml/ L respectively compare with 9.0 insect/ leaflet in control treatment (water only) table (1). The statically analysis revealed significant different between control treatment and using insecticides in this study but the results did not revealed significant different between spinetoram by three concentrates and bifenthrin after (10) days of spraying table (1). Table (2) showed the high percentage efficacy of using pesticides against dubas bug after (10) days of spraying, was reached 90.7, 86.9,88.7 & 89.5% for spinetoram with concentrate 0.50, 0.75 &1.0 ml/ L and bifenthrin with concentrate2.0ml/ L respectively.

The field observation showed that spinetoram had low effect on the predators belong to coccinellidae which was spreading in Iraqi environmental, especially the concentrate 0.50 ml/ .L compare with high effect for bifenthrin. (5) found that spinetoram was high effect against *Aphis fabae* but low effect on the *Conccinella undicmpunctata*, the percentage of killing was less than 50% after (10) days from spraying and the predator female can lay eggs.also (15) found that spinetoram was high effective against the larvaeof *Pectinophora gossypiella* while (16)confirmed that spinetoram had low effect on the predators in the cotton field when it used concentrates . according to recommendation

CONCLUSION

Belong to above results, we can use spinetoram with concentrate 0.50 ml / L. because it gave the same efficacy for another concentrates in addition to reducing the control cost and represented new chemical group beside to low effect on predators .the using new insecticides from different chemical groups was very important to avoid the resistance which induced by dubas bug against insecticides .we suggested to conduct additional studies about the effective

of spinetoram on the the predaors which was found in the date palm orchards in order to use it whit in IPM program.

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Tables

Table (1): The average no. of date palm dubas bug *O. binotatus lybicus* before and after spraying by 3,7& 10 days.

Treatments	Average no. of insect before spraying	Average no. of insect after spraying by days		
		3	7	10
Spinetoram 12SC 0.50 ml/L	19.86	7.8	3.3	0.60
Spinetoram 12SC 0.75 ml/L	21.83	6.46	2.3	0.93
Spinetoram 12SC 1.0 ml/L	20.46	12.7	3.5	0.73
Bifenthrin (2 ml/L)	13.46	1.93	0.73	0.46
Control(water only)	29.6	12.7	9.0	9.6
LCD at 0.05	13.9	5.34	2.09	2.0

Table (2) – the percentage efficacy of spinetoram 12 sc against date palm dubas bug *O.binotatus lybicus* by using Henderson & Tilton equation .

Treatments	% efficacy by days		
	3	7	10
Spinetoram 12SC 0.50 ml/L	8.5	45.4	90.7
Spinetoram 12SC 0.75 ml/L	31.3	65.5	86.9
Spinetoram 12SC 1.0 ml/L	-35(0.0)	47.50	88.7
Bifenthrin (2 ml/L)	66.6	82.1	89.5

Density of date fruit (*Phoenix dactylifera* L.) suspension and clarified extract as influenced by concentration and temperature

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ABSTRACT

The present study was, carried out to investigate the effect of concentration and temperature on the density of date paste-water suspension and its filter press clarified extract produced from date fruit. The density was determined in the temperature range 5 to 70°C, and the concentration ranges 21 to 43 (w/w %) total solids for the suspension, and 10 to 70 °Brix for the clarified extract. Density consistently increased with the increase of concentration and decrease of temperature, and fell within the ranges 1097 to 1429 and 1009 to 1447 kg m^{-3} for the investigated date suspensions and clarified extracts, respectively. Predictive models were successfully, fitted to experimental data. Significance of main effects and interactions were statistically determined.

Key words: dates, physical properties, density, juice, paste, pumping, pipe transport, predictive models.

INTRODUCTION

The date fruit of date palm trees (*Phoenix dactylifera L.*) is the most grown fruit in the Kingdom of Saudi Arabia with an annual production of 1.08 million tons of dates from 400 different cultivars and 24 million date palm trees (Ministry of Agriculture, 2011). This production represents about 13.67 % of the total world production of 7.9 million tons of dates. Currently, the Kingdom is the second largest date producing country in the world preceded by Egypt (FAOSTAT, 2011). The date suspension is usually produced by mechanical mixing and moderate heating of date paste and water consequently allowing dissolved sugars and other soluble solids to be separated by filtration or centrifugation for the production of clarified date extract (Hassan and alhamdan, 2010; Alahmar, 2011). The separated clarified date extract have several potentials for industrial scale production of a range of date derivative products. Several investigators (Mustafa *et al.,* 1983; El-Shaarawy *et al.,* 1989; Hamad and Al-Besher, 1996; Al-Farsi *et al.,* 2007; Chaira *et al.,* 2007; Hassan, 2009) successfully produced natural date juices and carbonated date drinks. Natural date syrup (Mohamed and Ahmed, 1981; Ali *et al.,* 1996; Ramadan, 1998; Alharthi, 1999; Hassan, 2003; Entezari *et al.,* 2004; Alahmar, 2011); and high fructose date syrup and date liquid sugar (Hassan, 2003; Al-Twaijri, 2005; Gaily *et al.,* 2010) are also successful date derivative products. In addition, fermentation products such as date vinegar, medical and industrial alcohol, and baker's yeast were also investigated (Mehaia, 1991; Al-Abid, 2006; Hassan, 2009).

Density is one of the most important physical properties of liquid food needed in design, selection, operation, and modeling of a series of essential transporting and processing unit operations. Such unit operations include pumping and fluid flow, centrifugal separation, filtration, membrane separation, heat exchangers, pasteurization and sterilization, mixing and homogenization, evaporation, freezing, in addition to packaging systems and aseptic processing (Hassan and Alhamdan, 2010; Alahmar, 2011). The availability of

accurate date suspensions and clarified extracts density data and predictive mathematical models is highly needed. Presently such data are rare in the open literature.

Variations in density for fruit juices have been, reported to be a function of concentration and temperature (Constenla, *et al.,* 1989; Ramos and Ibarz., 1998; Cepeda and Villaran, 1999; Zainal *et al.,* 2000; Azoubel *et al.,* 2005). Shamsudin, *et al.,* 2005 reported that density of guava juice increase with an increase in concentration and with a decrease in temperature. The experimental values obtained were well fitted with the same equations proposed by Ramos and Ibarz (1998). Several studies (Constenla *et al.,* 1989; Telis-Romero *et al.,* 1998; Azoubel *et al.,* 2005; Zuritz *et al.,* 2005; Shamsudin, *et al.,* 2005) have reported mathematical models correlating density of fruit juices, soluble solids content, and temperature.

The objective of the present study was to investigate the influence of concentration and temperature on density of date suspension and clarified extract, and fit appropriate predictive mathematical models to experimental data.

MATERIALS AND METHODS

Date suspension and clarified extract preparation

Three popular Saudi date fruit cultivars, namely, Sukkari, Khnaizi and Khudari at Tamr stage of maturity, produced during the date production season 2009/2010, and not pre-treated chemically or mechanically, were secured from well-known date palm trees farms in Qassim region (Sukkari), Riyadh region (Khudari), and the Eastern region (Khnaizi). Date fruits were, washed with potable water, spread in trays openly at room temperature (23 °C) for 24 h to dry surface water. Date pits were, removed by a date pitting machine (Date pitting machine, Zallaly, Saudi Arabia) and the produced date flesh were ground in a pilot scale date grinder (VEKL 1- IEC-34, Italy) to produce date paste. A stainless steel tank with an attached mechanical mixer, a heating element, and a temperature controller allowed mixing the date paste with potable water in a ratio (1: 2.5 (w/w) date paste to water), to produce a homogeneous suspension. The mechanical mixing process involved a mixing temperature not exceeding 70°C, for 30 minutes, to produce a homogeneous date paste- water suspension (Alharthi, 1999; Hassan and Alhamdan, 2010; Alahmar, 2011). Part of the produced date suspension was filled into clean sterilized 1L glass jars with tight closure, and the other part was filtered using a pilot scale mechanical filter press (Filter press, Seitz Pilot, A20z, Germany) to produce the clarified date extract (juice) (Alharthi,1999; Hassan and Alhamdan, 2010; Alahmar, 2011). Part of the produced clarified date extract was concentrated to, 70°Brix in a pilot scale rising film natural convection evaporator under vacuum (QVF Teaching system, CTSY Evaporation, Climbing Film and Natural Circulation Evaporator, QVF, Germany) at a temperature not exceeding 70°C (Alharthi,1999; Hassan and Alhamdan, 2010; Alahmar, 2011). The produced single strength and vacuum concentrated clarified date extract were, filled into sterile tight closure glass jars. The date suspension, and single strength and concentrated clarified date extract samples were transferred to a freezer (-18°C), and kept frozen until performing the density measurement experiments.

Suspensions with different total solid contents were prepared as described above by mixing date paste and potable water in different ratios, namely, 1:1.5, 1:2.0, 1:2.5, 1:3.0 (date paste: water (w/w)). Total solids of the date suspensions were determined by vacuum oven (AOAC, 1995). The clarified date extracts samples were prepared through mass balance calculation. The total soluble solids (TSS) were determined using a digital refractometer (Bellingham + Stanley Limited, London, England)

The proximate chemical composition of the flesh of the three date cultivars at Tamr stage of maturity is adapted from Sawaya, et al. 1986, as shown in Table (1).

Density Measurement

Density of date fruit suspensions and clarified extracts were experimentally measured in six replications using a 50 ml volumetric glass pycnometer in the temperature range 5 to70°C using the procedure followed by Ramos and Ibraz, 1998, Tsen and King, 2002, and Souza *et al.,* 2009. A controlled temperature water bath (TC-502, Circulating Refrigerated Water Bath, Brookfield Engineering Laboratories, USA), and a 0.001 g sensitive balance (Mettler Toledo PG 203-S, Toledo Comp, Switzerland) were used in the density determination. The density of samples were calculated using the following equation (Souza *et al.,* 2009)

$$\rho = \rho_w .((m_s-m_v)/(m_w-m_v)) \qquad (1)$$

Where r is juice density ($kg.m^{-3}$), r_w is water density at juice temperature; m_s sample mass (kg), m_w mass of water (kg), m_v pycnometer mass (kg). Flow diagram of samples preparation and density determination procedure is depicted in Fig.1.

Data Analysis

PASW for Windows software, version18 (IBM Co., NY, USA), has been used to carry out the statistical model fitting and ANOVA. The suitability of the fitted models was, evaluated by determination coefficient (R^2), and the significance level.

RESULTS AND DISCUSSION

Concentration effect on density

Density was directly proportional to total solids (TS) of suspensions and total soluble solids (TSS) of clarified extracts for the three date cultivars as shown in Fig. 2. In comparison with other studies, the values of density obtained for the clarified extracts are close to those obtained by Ramos and Ibarz (1998) for peach and orange juices. On other hand, there is rare published data to compare with density of date fruit suspensions.

The experimental data of density of date fruit suspensions and clarified extracts were, successfully fitted to a linear model predicting density as a function of concentration at constant temperature, as shown in Table 2. As a result, all models has high coefficient of determination (R^2) at a probability level of 95%.

Temperature effect on density

The experimental data obtained for the density of the suspensions and clarified extracts of the three date cultivars at the studied concentrations and temperatures are, shown in Fig. 3. As was expected, density is inversely proportional to temperature, which agrees well with many studies (Ramos and Ibarz, 1998; Cepeda and Villar, 1999; Tsen and King, 2002; Zuritz *et al.,* 2002). Linear and Arrhenius-type models well fitted the density data of both suspensions and clarified extracts as shown in Tables 3 and 4. As a result, the experimental data has high coefficient of determination at a probability level of 95%.

Density as a function of Concentration and temperature

Although there are several empirical models that were used by researchers to estimate the density of fruit juices in terms of temperature and concentration (Assis *et al.,* 2006; Tadini *et al.,* 2005; Gratao *et al.,* 2005), this study selected the most common form where density varies linearly with temperature and second order polynomial with concentrations. Ramos and Ibarz, 1998, and Tsen and King, 2002, as shown in the following equation, used this form:

$$r = a_1 + a_2 T + a_3 C + a_4 C^2 \quad (2)$$

Where r is the density in kg m^{-3}, T is temperature in °C, C is the suspension total solids (w/w %) and juice total soluble solids in °Brix; and a_1-a_4 are the model coefficients.

The estimated parameters and the determination coefficient for the proposed model for each date cultivar are, tabulated in Table 5. The result shows that the predictive model well fitted the experimental data for each of the date fruit cultivars. The same predictive model fitted all experimental data of density of the three date fruit cultivars suspensions and clarified extracts and showed acceptable coefficients of determination, namely, R^2=0.835 for the suspensions, and R^2=0.879 for the extracts.

Figure 4 illustrates the response of density to temperature and concentration.

Analysis of variance

Factorial analysis of variance was, carried out to investigate the significance of the effect of date fruit cultivar, concentration and temperature, and their interactions on suspension and clarified extract density. Tables 6 and 7 show the ANOVA results for the suspension and clarified extract, respectively. The main effects (date fruit cultivar, concentration (TS and TSS), and temperature (T), showed significantly differing effects at the probability level of 95%, whereas all interactions were insignificant for date suspension density, and were significant for date clarified extract density except cultivar-temperature interaction.

CONCLUSIONS

Linear predictive models successfully fitted experimentally determined density of date fruit suspension and clarified date extract, as a function of concentration at constant temperature, and temperature at constant concentration, in addition to an Arrhenius-type model as a function of temperature at constant concentration. A second order polynomial equation successfully fitted the data, and can predict density of date suspension and clarified date extract as a function of both concentration and temperature. These predictive models might be useful for equipment and process design, simulation analysis, and better control of products and treatments.

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Tables:

Table 1: Proximate composition (g/100 g dry matter) of the flesh of date cultivars Sukkari and Khudari (Hassan *et al.,* 2014) and Khnaizi (Sawaya *et al.,* 1986) atTamr stage of maturity.

Component	Sukkari	Khudari	Khnaizi
Moisture	31.11±0.51	23.56±1.97	16.20
Crude Protein	3.37 ±0.05	2.40±0.01	2.50
Total Fat	0.14 ±0.01	0.16±0.03	0.20
Crude Fiber	4.13 ±0.23	3.28±0.17	2.40
Ash	1.65 ±0.18	1.83±0.11	1.50
T. Carbohydrate	90.70 ±0.36	92.33±0.13	---
Total Sugars	79.83 ±1.46	91.03±1.24	74.00
Fructose	7.52 ±0.16	45.40±2.53	36.26
Glucose	8.90 ±0.59	45.14±1.76	37.74
Sucrose	63.4 ±0.75	0.49±0.14	0.00
TSS (°Brix)	76.23^{a} ±0.55	83.00^{b}±0.72	---

Table 2: Constants of predictive models for date fruit suspension and clarified extract density as influenced by concentration at constant temperature.

Variety	T, °C	$\rho=k_1+k_2\times TS^*$			$\rho=k_3+k_4\times TSS^*$		
		Suspension			Clarified extract		
		k_1	k_2	R^2	k_3	k_4	R^2
Sukkari	5	857.559	13.086	0.943	985.571	6.314	0.978
	15	866.359	12.133	0.938	984.286	6.182	0.979
	25	880.799	11.070	0.950	981.000	9.093	0.977
	35	886.316	10.133	0.944	977.714	6.025	0.977
	45	900.407	9.149	0.962	975.000	5.946	0.977
	55	900.500	8.560	0.963	971.714	5.868	0.976
	70	930.364	6.601	0.975	964.857	5.861	0.977
Khnaizi	5	701.478	17.726	0.987	999.000	6.157	0.990
	15	725.528	16.429	0.987	996.286	6.032	0.990
	25	753.755	14.967	0.987	994.429	5.857	0.987
	35	785.021	13.505	0.988	990.000	5.814	0.990
	45	810.267	12.043	0.988	987.000	5.700	0.989
	55	842.571	10.437	0.990	987.000	5.586	0.988
	70	880.426	8.395	0.989	979.714	5.407	0.984
Khudari	5	928.758	12.217	0.976	1037.857	3.443	0.992
	15	916.125	12.149	0.981	1031.429	3.493	0.994
	25	903.491	12.081	0.985	1023.571	3.568	0.995
	35	890.859	12.013	0.988	1016.143	3.636	0.966
	45	878.225	11.945	0.991	1009.286	3.693	0.996
	55	0.95.592	11.877	0.994	1002.429	3.750	0.997
	70	845.825	11.796	0.997	991.429	3.846	0.998

* ρ= density, kg m^{-3}; T = temperature, °C; TS= total solids, (w/w) %; TSS= total soluble solids, °Brix; k_1 – k_4=constants.

Table 3: Constants of predictive model for date fruit suspension and clarified extract density as influenced by temperature at constant concentration.

Variety	TS*, %	$\rho=k_5 + k_6 \times T^*$						
		Suspension			TSS*, °Brix	Clarified extract		
		k_5	k_6	R^2		k_5	k_6	R^2
Sukkari	42.78	1445.002	-3.256	0.999	10	1042.840	-0.476	0.998
	33.39	1300.865	-2.092	0.998	20	1107.625	-0.505	0.994
	30.50	1229.876	-1.573	0.979	30	1199.000	-0.400	0.997
	24.75	1213.352	-1.634	0.991	40	1261.000	-0.600	0.997
					50	1286.982	-0.651	0.993
					60	1337.000	-0.600	0.997
					70	1448.224	-0.994	0.989
Khnaizi	39.39	1422.167	-2.993	0.984	10	1064.000	-0.600	0.995
	32.72	1287.636	-1.886	0.992	20	1108.375	-0.495	0.994
	29.14	1209.625	-1.305	0.987	30	1211.625	-0.705	0.996
	25.30	1168.000	-1.001	0.991	40	1249.375	-0.495	0.994
					50	1304.625	-0.705	0.990
					60	1357.970	-0.871	0.981
					70	1444.000	-1.393	0.990
Khudari	35.12	1375.000	-1.600	0.994	10	1075.263	-0.861	0.993
	29.10	1274.625	-1.305	0.985	20	1105.380	-0.607	0.994
	24.31	1233.000	-1.400	0.979	30	1147.625	-0.505	0.999
	21.23	1202.625	-1.505	0.991	40	1174.000	-0.400	0.987
					50	1218.625	-0.505	0.992
					60	1255.000	-0.400	0.982
					70	1270.000	-0.200	0.987

*ρ= density, kg m^{-3}; T = temperature, °C; TS= total solids, (w/w) %; TSS= total soluble solids, °Brix; k_5 and k_6=constants.

Table 4: Constants of Arrhenius-type model for date fruit suspension and clarified extract density as influenced by temperature at constant concentration.

Variety	TS* %	$\rho = \rho_0 e^{(E_a/RT)}$*						
		Suspension			TSS* °Brix	Clarified extract		
		ρ_0	E_a	R^2		ρ_0	E_a	R^2
Sukkari	42.78	619.98	1944.99	0.990	10	888.90	366.16	0.990
	33.39	721.51	1353.75	0.993	20	943.87	366.80	0.994
	30.50	774.42	1061.83	0.994	30	1067.20	267.06	0.996
	24.75	744.73	1120.53	0.994	40	1067.10	382.92	0.995
					50	1092.70	380.21	0.986
					60	1142.30	360.79	0.996
					70	1134.80	559.67	0.998
Khnaizi	39.39	648.39	1804.97	0.992	10	872.39	455.30	0.995
	32.72	755.60	1223.80	0.992	20	947.81	358.85	0.997
	29.14	821.25	888.64	0.994	30	986.96	470.30	0.994
	25.30	861.22	699.01	0.995	40	1087.70	317.75	0.997
					50	1078.70	436.09	0.994
					60	1082.10	520.90	0.997
					70	1023.50	790.75	0.995
Khudari	35.12	904.44	961.39	0.994	10	869.54	487.02	0.996
	29.10	883.31	841.50	0.994	20	911.25	442.88	0.995
	24.31	819.66	937.03	0.994	30	983.51	353.81	0.994
	21.23	765.22	1037.72	0.994	40	1042.30	272.81	0.996
					50	1053.90	332.88	0.994
					60	1122.90	254.98	0.996
					70	1202.50	125.24	0.996

*ρ= density, kg m^{-3} ; r_0= constant, kg m^{-3} ;E_a= activation energy, j $gmol^{-1}$; R=8.3144 j $gmol^{-1}K^{-1}$= universal gas constant; T = temperature, K; TS= total solids, % (w/w);TSS= total soluble solids, °Brix.

Table 5: Constants of predictive models of density as a function of temperature and concentration, for the suspensions and clarified extracts of date varieties.

Variety	$\rho = a_1 + a_2T + a_3C + a_4C^{2*}$						
	a_1	a_2	a_3	a_4	R^2	Temperature °C	TS,% (w/w)
	Suspension						
Sukkari	1291.016	-2.139	-9.737	0.291	0.955	5-70	24.75-42.78
Khnaizi	1166.021	-1.453	-4.425	0.290	0.977		25.30-39.38
Khudari	1129.062	-1.796	-4.296	0.271	0.957		21.23-35.12
ALL	1148.876	-1.796	-1.254	0.174	0.835		21.23-42.78
	Clarified extract						TSS,°Brix
Sukkari	997.309	-0.752	7.101	-0.016	0.989		10-70
Khnaizi	1024.923	-0.466	4.150	-0.006	0.995		
Khudari	982.912	-0.604	7.096	-0.013	0.978		
ALL	1000.883	-0.663	6.358	-0.016	0.879		

* ρ= density, kg m^{-3}; T = temperature, °C; C= TS= total solids (% (w/w)), for suspension; C=TSS= total soluble solids (°Brix), for clarified extract; $a_1 - a_4$=constants.

Table 6: ANOVA results for date fruit suspension.

Source	df	Type III SS	MS	F	P
Main Effects					
Variety	2	34840.5	17420.3	4.062	0.019
TS	3	1117476.7	372492	86.85	0.000
Temperature	6	517827.7	86304.6	20.12	0.000
Interaction					
Variety * TS	6	21924.4	3654.1	0.852	0.532
Variety * Temperature	12	60706.0	5058.3	1.180	0.301
TS * Temperature	18	101336.3	5629.8	1.313	0.185
Variety * TSS * Temperature	36	177175.1	4921.532	1.147	0.277
Error	168	720540.0	4288.929		

Table 7: ANOVA results for date fruit clarified extract.

Source	df	Type III SS	MS	F	P
Main Effects					
Variety	2	254384.2	127192.1	65.9	0.000
TSS	6	3300771.3	550128.6	285.3	0.000
Temperature	6	52859.8	8809.9	4.568	0.000

Interaction					
Variety * TSS	12	381961.6	31830.1	16.505	0.000
Variety * Temperature	12	27122.9	2260.2	1.172	0.302
TSS * Temperature	36	296583.3	8238.4	4.272	0.000
Variety * TSS * Temperature	72	345531.0	5081.3	2.635	0.000
Error	294	586276.0	1928.5		

Figures:

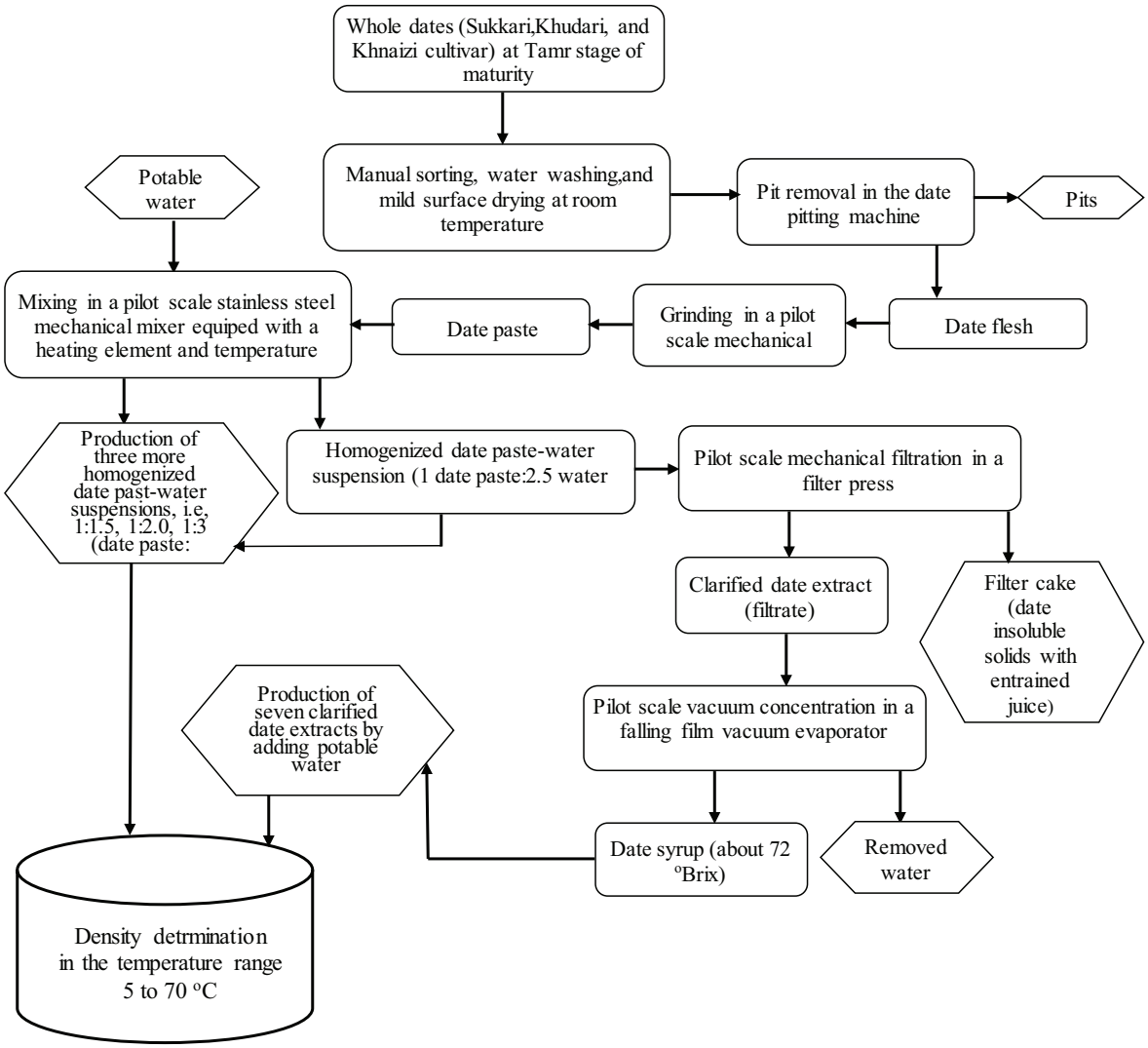

Fig.1. Flow diagram of samples preparation and density determination procedure of homogeneous date paste-water suspension and its clarified extract.

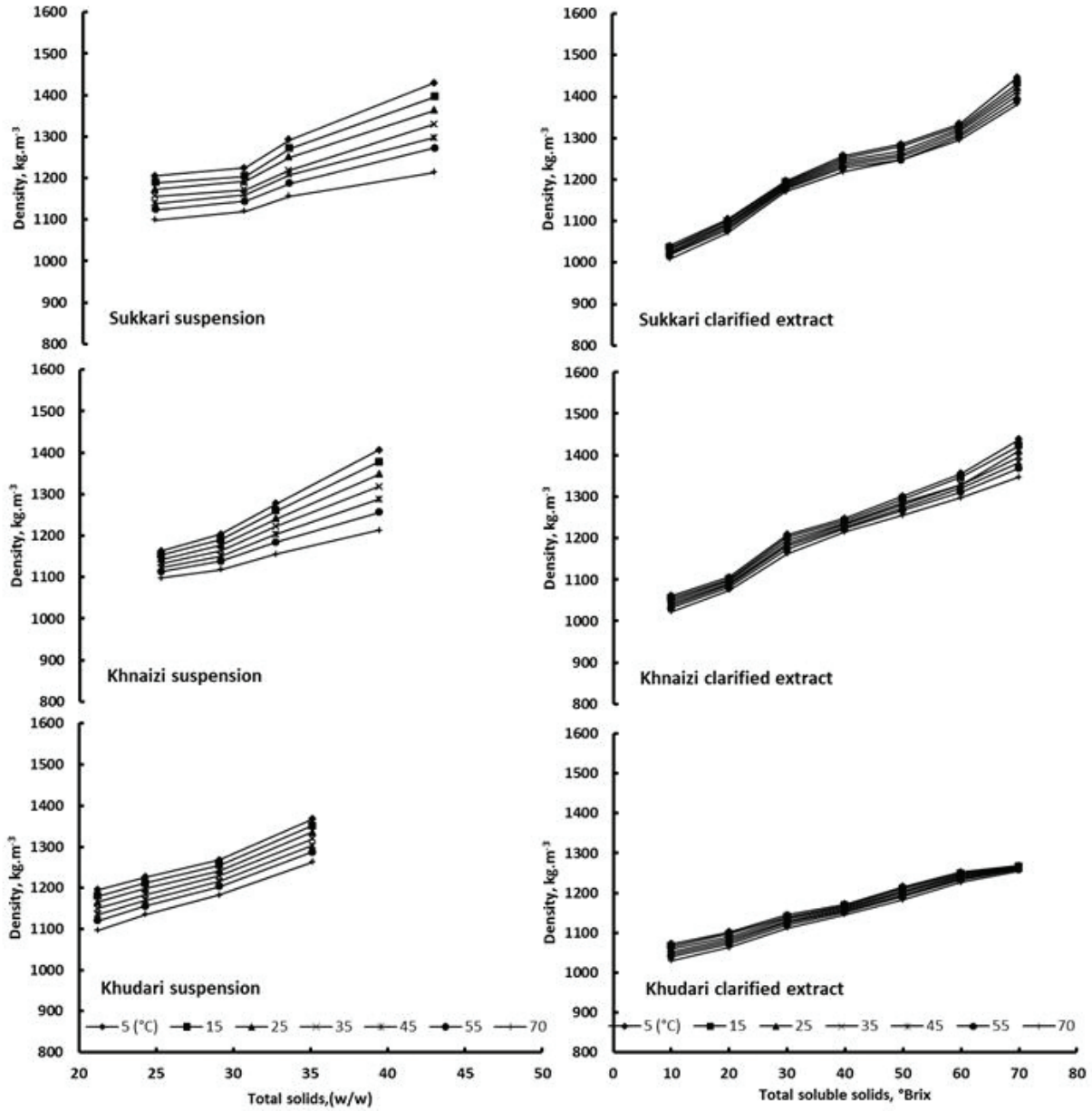

Fig. 2. Effect of date fruit suspension total solids and clarified extract total soluble solids on density at different temperatures.

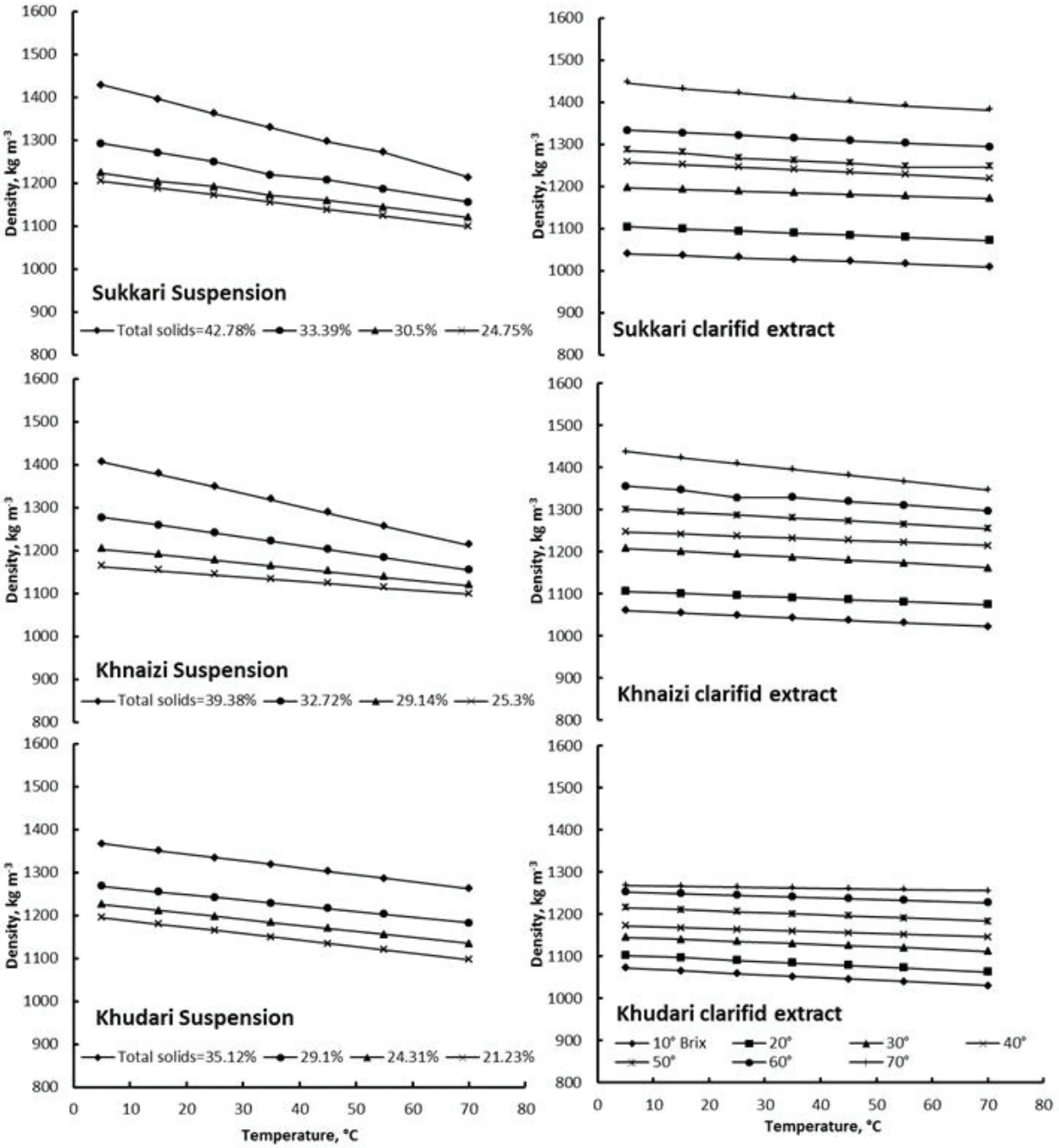

Fig. 3. Effect of date fruit suspension and clarified extract temperature on density at different solids concentrations.

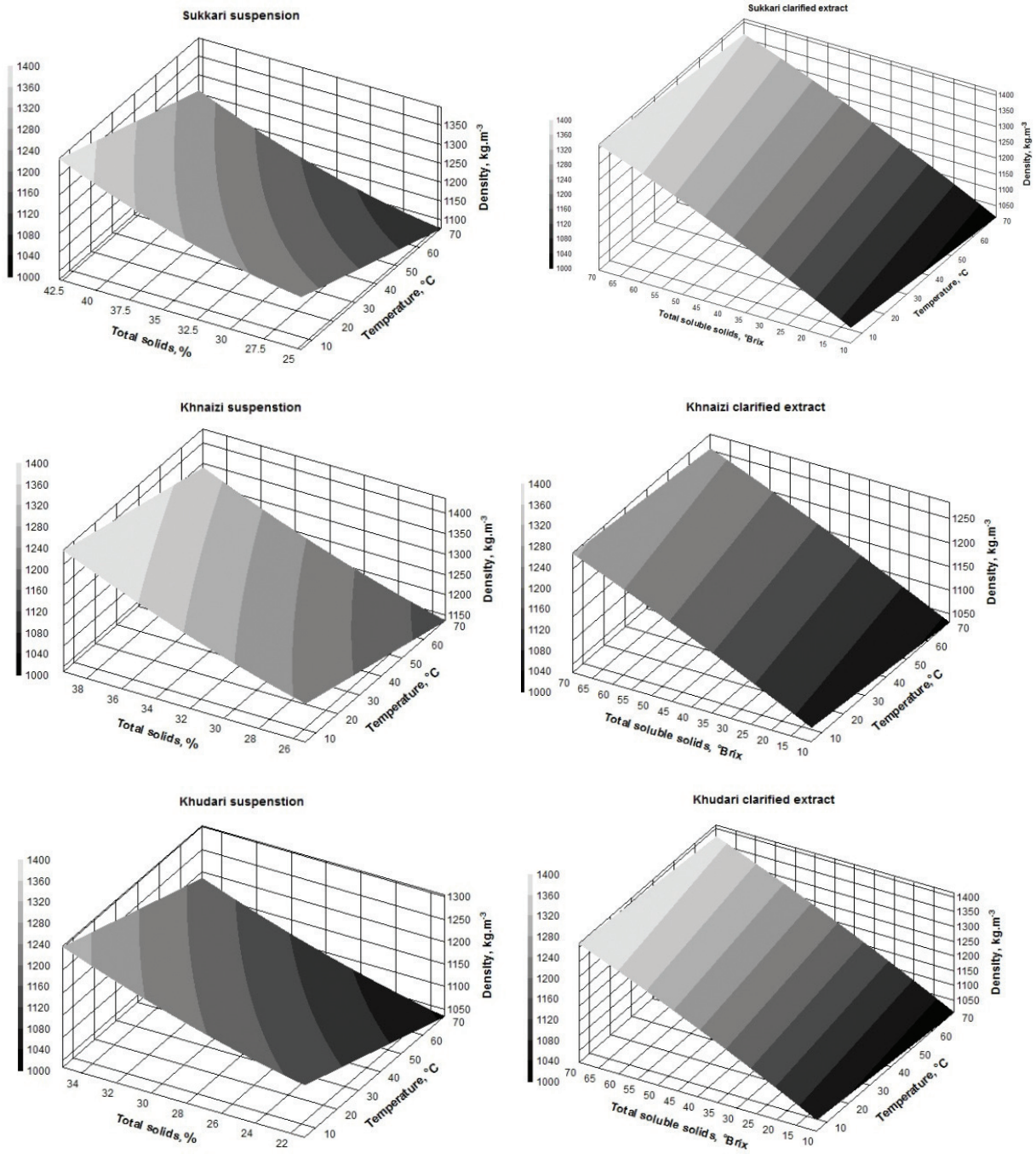

Fig. 4. Density response surface as a function of temperature and concentration.

ANN modeling of water sorption isotherm of date fruits acquired by dynamic vapor sorption

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ABSTRACT

Water sorption isotherms of four Date cultivars at temperatures range of 10 to 80°C with increments of 10°C were determined separately over ranging relative humidity from 10 to 90% using an automatic, gravimetric analyzer. The significant differences in equilibrium moisture contents (EMC) of cultivars can be attributed to chemical composition and structure of each cultivar. The adsorption / desorption experimental data for each cultivar were fitted by modified GAB, modified Halsey, modified Smith and modified Oswin models. The modified Oswin was found as best model to describe adsorption isotherm behavior while modified Halsey was the best model to describe desorption isotherm. In addition, many configurations of artificial neural networks (ANN) were examined to obtain the best one to predict either EMC adsorption isotherm or EMC desorption isotherm for each cultivar. Correlation coefficient (R), c^2, root mean square error (RMSE) and relative percent errors (PE) were used to measure the performance of the four models as well as ANN model. The ANN model was able to predict EMC over the whole ranges of temperature and relative humidity with an R ranging from 0.9878 to 0.9999. A user-friendly interface program was created to predict EMC based on ANN model as well as the other four models.

1. INTRODUCTION

Prediction of shelf life stability of fruits or design and optimization of processing require knowledge and understanding of changes that takes place. Those changes are influenced mainly by the moisture content and water activity of fruit material (Jamali *et al.,* 2006). The association between equilibrium moisture content (EMC) and water activity (aw) at constant temperature and pressure is defined as moisture sorption isotherm (MSI) (Alp *et al.,* 2009; Bell and Labuza, 2000; Rahman, 1995). In addition, Berg and Bruin (1981) stated that understanding MSI is one of keys to develop new food products with optimal safety and quality attributes. For each food type, the MSI shape is unique due to differences in its structure and composition effects. For most foods, the isotherm is sigmoidal in shape, although foods that contain large amounts of sugar or small soluble molecules have a J-type isotherm curve (Kapsalis, 1987; Bruin and Luyben, 1980).

The three well-known isotherm methods which classically used for most sample types are: Traditional Desiccator Method (TDM), Dynamic Vapor Sorption (DVS) and Dynamic Dewpoint Isotherm (DDI) method, the advantage and disadvantage of each method were quantified and discussed in (Gokhan, *et al.,* 2012; Brady and Anthony, 2008; Rahman and Al-Belushi, 2006). However, they claimed that for samples that practice a phase change during sorption measurement or have slow diffusion properties, the results may vary.

A significant amount of theoretical, empirical or semi-empirical correlations has been mentioned in the literature to estimate the equilibrium moisture of biological materials. Van den Berg and Bruin (1981) reported that there are more than 200 EMC/ERH equations are available for foods; however,

no single equation has the ability to describe accurately the EMC/ERH relationships for various grains over a broad range of relative humidity and temperature. In addition, temperature level affects isotherms, and some models considers this factor (Sun and Woods, 1993; Wang and Brennan, 1991; Chen and Morey, 1989; and Chirife and Iglesias, 1978).

Although the massive effort that has been done on developing equilibrium isotherms for different biological materials, very limited literature was found on the sorption and desorption characteristics of date fruits (Ferradji, *et al.,* 2012; Belarbi, *et al.,* 2000; Myhara, *et al.,* 1998; and Hassan, 1991),characteristics of date pastes (Alhamdan and Hassan, 1999) and characteristics of date powder (Hassan, 2002)

Date palm is one of the major agricultural crops of the Near East region, where about 90% of the world dates production takes place. The date fruits play an important economical and sociological part in many countries in this region. About 450 cultivars are grown in SA, among these 60 cultivars are well known, date fruits have several stages of maturity they are conventionally described by changes in color, texture and taste/flavor. The date fruit *(Phoenix dactylifera L.)* is a much-appreciated fruit, not only for its refreshing taste, but also for its nutritional qualities. (Hassan, 1991).

Many researcher in field of food engineering use ANNs technique to solve either prediction or classification problems, most of published studies prove the importance and wide acceptability of this technique in solving such problems. Because of the nature of its nonlinear structure, ANNs are particularly useful for detecting complex underlying relationships, which found in many real world problems (Alhassan and Misra, 2011).

In the problem of moisture sorption isotherms prediction, many researchers utilize ANNs to predict this behavior for many food materials (Hamid and Mohsen, 2013 for pistachio powder; Al-Mahasneh *et al.,* 2012 for roasted green wheat; Mohammad et al. 2012 for some of agricultural material include dates; Gazor and Eyvani 2011 for red onion slices; Chayjan and Esna-Ashari 2010 for raisin; Chayjan 2010 for sesame seed; Paulo *et al.,* 2010 for coffee; Janjai *et al.,* 2009 for longan fruit; Chayjan and Moazez 2008 for three cultivars of paddy; Guilan *et al.,* 2007 for cornstarch; and Robert and Sablani 2001 for ten agricultural materials include date fruits).

Hamid and Mohsen (2013) tested several mathematical models to predict the moisture sorption isotherm of pistachio powder; they proved that Caurie model was the most suitable one. In addition, artificial neural network approach was used. The results showed that, ANN could able to predicted adsorption-desorption moisture content with R^2 values 0.998 and 0.992, respectively. Comparison of ANN results with conventional sorption isotherm models showed that ANN modeling had greater accuracy to predict EMC of pistachio powder.

Al-Mahasneh *et al.,* (2012) use both of neural-fuzzy technique and four mathematical models, which are modified Halsey, modified Oswin, modified Henderson, and modified Smith in fitting experimental data from moisture sorption isotherms of roasted green wheat. Compared with empirical models, neural-fuzzy modeling provided a much better prediction for moisture sorption isotherms as showed by statistical measures.

Gazor and Eyvani (2011) practice conventional mathematical models and ANNs to find out the moisture sorption isotherms of red onion slices at temperature ranged between 30 to 60 °C using the standard gravimetric static method over a range of relative humidity from 11% to 83%. In Mathematical models, modified Oswin model was better than others. As well, an ANN model with two inputs (temperature and relative humidity), one output (equilibrium moisture content) and two hidden layers was found to be able to predict the equilibrium moisture content after it was adequately trained.

Chayjan and Esna-Ashari (2010) use six empirical models and ANNs for predicting of (EMC) in raisin. The results show the power of ANN, in compare with empirical models. The study suggested that, the empirical models could be replaced with the ANN model.

Paulo et al. (2010) perform desorption isotherms properties of coffee from different processing stages during the drying. The isotherms were determined by a static gravimetric method for various temperature and humidity conditions. Several mathematical models in addition to ANN model correlated EMC data. Based on statistical parameters, the ANN model, modified Henderson and GAB models were adequate to describe the sorption characteristics of the samples.

Janjai et al. (2009) developed ANN model to predict the EMC of longan fruit (*Dimocarpus longan Lour*). After proper train, ANN model is found be better than the well-known Henderson, and GAB models. They concluded that as the ANN model predicts EMC more accurately, therefore better equations for heat of sorption and entropy could be developed based on data from the ANN model.

Guilan et al. (2007) determine the adsorption and desorption isotherms for corn starch powders using a gravimetric technique, then the obtained data were fitted to Halsey, Oswin, Henderson, Modified-BET, GAB, Peleg and ANN. Analysis showed that, within the investigated temperature range, GAB, Peleg and Henderson models better describes the experimental data for corn starch powder.

Robert and Sablani (2001) determine equilibrium moisture content for ten selected fruits experimentally. They concluded

that unlike the GAB equation, which uses only physical data for modeling, the ANN method uses both physical and chemical compositional data to do the predictions. In addition, the ANN model was able to show a temperature dependent crossing of water sorption isotherms, due to the dissolution of sugar crystals in the fruit. The ANN was also able to predict the extent of crossing, depending on di erences in the individual fruit chemical composition.

The purpose of the present study is to experimentally obtain water sorption isotherms for four date fruits cultivars to understand their water behavior in the temperature range of 10 to 80 °C and relative humidity range of 10 to 90%, and correlating experimental data with well-known modified GAB, modified Halsey, modified Smith and modified Oswin models. Additionally, to examine the ability of different ANNs configuration to predict adsorption-desorption moisture content of the selected date fruits cultivar.

2. MATERIAL AND METHOD

2.1 Sample preparation

Four date fruits cultivars were selected for their importance among all cultivars in SA, those cultivars were Sukkari, Saqie, Khudari and Khlass. Random fruits were picked and pits were manually removed using a sharp knife by cutting the fruit into two longitudinal halves. The date flesh was, carefully cut into small pieces less than one cm long, retaining both the external and internal surfaces of the flesh. The cut flesh pieces were dried in a vacuum oven at about 200 mm Hg vacuum and 65 °C for 48 h or until a constant weight was achieved. The bone dried date pieces were carefully, kept in tightly closed glass jars at 5.0±0.5 °C prior to sorption experiments.

2.2 Dynamic Vapor Sorption

The moisture adsorption and desorption at different humidity (10-95%) and temperature (10-80°C) were measured using an advanced fully automated, gravimetric, dynamic vapor sorption (DVS) instrument (Aquadyne DVS, Quantachrome instruments, Boynton Beach, Florida, USA). The instrument is equipped with two ultrasensitive microbalances which can detect changes in the mass during the adsorption and desorption cycle. The required relative humidity was, generated by mixing dry nitrogen and distilled water saturated nitrogen flows, in the corresponding proportion using mass flow controllers and a calibrated humidity probe. The instrument was calibrated using microcrystalline cellulose (CRM # 302) dry powder. Pre-dried date flesh pieces samples (bone dried) were 80±5 mg in each of the two ultrasensitive microbalances. The software, which provides with the instrumentation, allows real time evaluation and export data through PC-instrument RS 232 interface. Mass, temperature, and humidity data were, recorded in 30 s time intervals. Equilibrium was, considered to have been, reached when the change in mass was less than 0.001 mg/min.

2.3 Isotherm Model

Among all isotherm models, four isotherm models that integrated temperature effects were tested for their widely use. The models were Modified Halsey, Modified Oswin, Modified GAB and Modified Smith as follows:

Modified Halsey model:

$$M = \left(-exp(A + BT)/ln(H_r)\right)^{1/C}$$

Modified Oswin model:

$$M = (A + BT)(H_r/(H_r))^{1/C}$$

Modified GAB model:

$$M = A(C/T)BH_r/(A(C/T)BH_r)$$

Modified Smith model:

$$M = (A + B) - [(C + DT)ln(1 - H_r)]$$

Where: M is the equilibrium moisture content (% d.b.), H_r is the equilibrium relative humidity (decimal), A, B, C, D are individual model dependent empirical constants and T is the temperature (°C).

The curve fitting toolbox 3.4 provided with MATLAB® R2013b (The MathWorks Inc., Natick, MA, USA) was used to fit experimental data to the selected four isotherm and determined constants. The curve fitting toolbox procedure use the Gauss-Newton algorithm with Levenberg-Marquardt method to solve the models. Performance of the isotherm models was evaluated using performance parameters for non-linear models, such as mean relative percent error, standard error, and correlation R (Viswanathan *et al.,* 2003; Menkov, 2000; and Chen and Morey, 1989).

2.4 ANN Modeling

Many studies related to ANNs application claimed that, there is no optimum structure for any problem, the optimum structure could be found by experience or try and error (Raju and Begum, 2013; Lendaris, 2004; and Cho *et al.,* 2000)

Prediction of EMS for each cultivar individually and for all cultivars is accomplished using multilayered feed forward network using standard back propagation method. Multilayered feed forward network is known as a supervised network because it requires a desired output in order to learn (Ripley, 1994).

In this research work, training ANN models completed using commercially available software Qnet 2000 (Vesta

Services, 2000). The software also provide a Dynamic Link Library (DLL) file, which can be used to send and receive data from the trained neural networks within the Visual Basic programming environment (Brown, 2005). In this research work, one and two hidden layers with 2-10 neurons per hidden layer, learning rate equal to 0.3, momentum coefficient equal to 0.9, activation functions of sigmoid, hyperbolic tangent and Gaussian in each hidden output layer and training iteration (epoch) equal to 5,000; 10,000 and 100,0005000 were used in order to find the best ANN configuration.

2.5 Data manipulation

The data sets were divided into three different sets named training, testing and validation sets. The training set is the largest set and is used by neural network to learn patterns present in the data. The testing is used to evaluate the generalization ability of supposedly trained network. A final check on the performance of the trained network was made using validation set. For each cultivar, 288 data sets used for the proposed architectures, 216 sets of them used for training process, 40 sets used for test process while the rest of data sets didn't involve to be used in model validation. The data for each set (training, test and validation) selected randomly. For the general model, 576 sets were used in developing model that cover all cultivars. Five hundred sets used as training set, 50 sets as a test while the rest, which equal to 26 sets used for validation process.

The input layer of proposed architectures for consisted of two variables, which are temperature and relative humidity while the output layer is EMC. To standardize data, the input and output values were normalized between 0.15 and 0.85 prior to use with the model, according to the following equation:

$$X(t) = \frac{(t - t_{min})}{(t_{max} - t_{min})} \times (0.85 - 0.15) + 0.15$$

Where: t is the original values of input or output variables, X (t) is normalized value, and t_{max} and t_{min} are maximum and minimum values of input or output variables.

A graphic user-friendly interface (GUI) was programmed using Microsoft Visual Basic® 6.0 (VB) to provide the user with an intuitive way of interacting with the application. The main purpose of GUI is to make the use of the application simple and quick. GUIs include a set of object buttons, text boxes and scroll bars that users use to work with applications and text elements that display information to the users. However, the behavior of those objects controlled by VB scripting language associated with each one. Then, the associated Dynamic Link Library (DLL) file from Qnet and the acceptable well-trained ANN used to predict EMC through GUI.

2.6 Model performance

The agreement between the predicted data and experimental data for all models is calculated through four key performance indicators (KPIs). The four KPIs were correlation coefficient; root mean square error; Chi- square; and the relative percent errors. Those KPIs can be calculated as follows:

Correlation of coefficient (adapts from Raquel, 2010):

$$R = 1 - \left(\sum_{i=1}^{N}\left(EMC_{pre,i} - EMC_{exp,i}\right)^2 / \sum_{i=1}^{N}\left(\overline{EMC_{pre}} - EMC_{exp,i}\right)^2\right)$$

Chi- square (adapts from Babalis *et al.,* 2006):

$$\chi^2 = \sum_{i=1}^{N}\left(EMC_{exp,i} - EMC_{pre,i}\right)^2/(N - n)$$

Root mean square error (adapts from Kingsly and Singh, 2007):

$$RMSE_{=}\sqrt{\left({}^{1}/_{N}\right)\sum_{i=1}^{N}\left(EMC_{pre,i} - EMC_{exp,i}\right)^2}$$

The relative percent errors (adapts from Roberts *et al.,* 2008):

$$PE = \frac{100}{N}\sum_{i=1}^{N}\left(\left|EMC_{exp,i} - EMC_{pre,i}\right|/EMC_{exp,i}\right)$$

Where: $EMC_{exp,i}$ is the equilibrium moisture content observed experimentally for instant i; $EMC_{pred,i}$ is the predicted equilibrium moisture content for instant i for the same instant N;N is the number of observations and n is the number of model constants.

Those KPIs used to describe the accuracy of models by many researches (Saidur and Masjuki, 2008; Dawson *et al.,* 2002; Loague and Green, 1991; and Nash and Sutcliffe, 1970). Model with the highest R and the lowest other KPIs values is the most suitable model (Minaei, *et al.,* 2012).

3. RESULTS AND DISCUSSION

3.1 Water sorption isotherm

The moisture adsorption – desorption isotherms for the four cultivars at different temperatures are shown in Figure (1) and Figure (2) respectively. Adsorption curves of all cultivars with a more or less pronounced sigmoidal and type-III shape where this is the general shape for materials with high sugar content (Tsami *et al.,* 1990). The figure showed that date fruits adsorb small amounts of water at low water activities and present a sharp increase in the quantity of adsorbed water at higher water activities. This behavior is typical of products with high sugar contents and several works reported similar results for tropical fruits (Telis *et al.,* 2000; Gabas *et al.,* 1999; and Hubinger *et al.,* 1992). EMC inclined to decrease with increasing temperature in a given water

activity for Saqie, Khudari and Khlass, while Sukkari behave as shown in Figure 1. This could be due to chemical and physical changes induced by temperature. The extent of this decrease depends on the composition of foods (Rizvi, 1995).

3.2 Isotherm Mathematical Models

The experimental data were fitted to the four isotherm models, which consider the influence of temperature. Estimated parameters and statistical results for adsorption behavior are shown in Table 2 while Table 3 was for desorption behavior. Although R^2 was ranged 0.934 to 0.960, the modified Oswin adsorption model presented relatively high root mean square error, and lower R^2 for all cultivars 0.842. On other hand, modified GAB was the best model that characterize desorption behavior for date cultivars.

3.3 ANN model

To obtain an optimum ANN model, minimal structure and minimal errors in training and testing sets were proposed (Sh. Youssefi *et al.,* 2009). Based on these criteria, the most suitable neural network structure with the minimum number of neurons in hidden layers to correlate the input and output parameters was selected as two hidden layer with four neurons in first hidden layer and two neurons in the second hidden layer using sigmoid function as activation function . For this structure, Fig 3 shows the observed-predicted relation for validation data set.

However, to avoid overfitting, the number of epochs was limited to 10,000. Increasing the epoch size may increase the problem of overfitting (Razmi-Rad *et al.,* 2007)

4. CONCLUSIONS

Based on the results of this research work it can be conclude that although some mathematical models could have good EMC prediction for the selected four date cultivars, ANN model could achieved EMC more precisely. Additionally, the study result in that the overall ANN model could be used in the range of 10-80°C accurately regardless of date cultivar. It is recommended that more cultivars could be tested within the general ANN model and based on test results ANN model may need re-train.

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Tables

Table 1: Adsorption isotherm models estimated parameters and statistical results.

Model	Parameters	Khlass	Khudari	Saqie	Sukkari	Overall
MGAB	A	11.460	18.30	8.525	4.527	9.813
	B	0.976	0.9238	0.994	1.006	0.9655
	C	74.47	26.03	81.73	147.20	73.13
	R^2	0.958	0.961	0.918	0.860	0.830
	SEE	0.1778	0.1681	0.2491	0.3468	2.479E-04
	RMSE	5.076	4.936	6.009	6.940	9.277

Model	Parameters	Khlass	Khudari	Saqie	Sukkari	Overall
MHalsey	A	2.393	2.601	1.955	0.834	2.218
	B	-0.001302	-0.007032	-0.0008995	0.009166	0.0009109
	C	1.018	1.017	0.9546	0.9016	1.06
	R^2	0.951	0.927	0.921	0.948	0.817
	SEE	0.2085	0.3163	0.2411	0.1280	2.662E-04
	RMSE	5.498	6.771	5.911	4.217	9.614
MSmith	A	-103.3	-75.55	-47.12	-47.45	-103.7
	B	93.52	65.47	38.02	37.37	94.49
	C	37.52	45.67	32.06	16.43	30.86
	D	-0.009261	-0.2118	-0.01614	0.1716	0.008094
	R^2	0.956	0.952	0.917	0.901	0.836
	SEE	0.1847	0.2069	0.2526	0.2464	2.295E-04
	RMSE	5.211	5.515	6.094	5.891	8.942
MOswin	A	14.82	17.91	11.28	3.205	11.28
	B	-0.01468	-0.09239	-0.0105	0.05769	0.009324
	C	1.2	1.205	1.122	1.02	1.227
	R^2	0.965	0.943	0.934	0.960	0.842
	SEE	0.1462	0.2479	0.2002	0.9813	2.387E-04
	RMSE	4.604	5.994	5.386	3.692	9.105

Table 2: Desorption isotherm models estimated parameters and statistical results.

Model	Parameters	Khlass	Khudari	Saqie	Sukkari	Overall
MGAB	A	17.02	33.73	14.82	10.07	18.50
	B	0.9063	0.7669	0.8896	0.9185	0.8549
	C	89.16	45.82	142.4	506.9	92.77
	R^2	0.961	0.954	0.928	0.9023	0.878
	SEE	0.1817	0.2056	0.1995	0.2240	1.787e-04
	RMSE	4.989	5.307	5.09	5.325	7.579
MHalsey	A	3.728	5.168	4.068	4.038	4.492
	B	-0.005416	-0.01541	-0.005746	0.0006643	-0.00711
	C	1.28	1.528	1.412	1.564	1.497
	R^2	0.941	0.920	0.913	0.894	0.845
	SEE	0.2713	0.3578	0.2394	0.2438	2.269E-04
	RMSE	6.096	7.001	5.575	5.555	8.542

Model	Parameters	Khlass	Khudari	Saqie	Sukkari	Overall
MOswin	A	-0.688	209.7	-83.67	-40.68	-83.70
	B	0.02558	-207.2	86.01	43.75	85.65
	C	38.44	47.1	31.94	21.19	34.39
	D	-0.1106	-0.3254	-0.1107	0.01879	-0.1295
	R^2	0.924	0.929	0.898	0.886	0.853
	SEE	0.3502	0.3165	0.2794	0.2623	2.147E-04
	RMSE	6.974	6.630	6.064	5.799	8.322
MSmith	A	24.07	36.17	23.68	16.77	25.65
	B	-0.07621	-0.2447	-0.08171	0.01106	-0.09666
	C	1.54	1.877	1.742	1.856	1.809
	R^2	0.941	0.925	0.910	0.887	0.850
	SEE	0.2717	0.3377	0.2491	0.289	2.191E-04
	RMSE	6.101	6.801	5.687	5.724	8.393

Table 3: Models Key Performance Indicators.

	Parameters	MGAB		MHalsey		MOswin		MSmith		ANN	
		Ads.	Des.	Ads.	Des.	Ads.	Des.	Ads.	Des.	Ads.	Des.
Khlass	R	0.924	0.935	0.962	0.966	0.932	0.901	0.944	0.902	0.999	0.999
	RMSE	0.517	0.654	0.425	0.444	0.661	0.724	0.743	0.865	0.054	0.052
	c^2	0.175	0.177	0.151	0.168	0.224	0.253	0.347	0.338	0.002	0.001
	PE	5.243	6.954	3.471	3.480	6.274	6.970	4.488	5.047	0.987	0.961
Khudari	R	0.942	0.941	0.967	0.958	0.942	0.944	0.914	0.911	0.989	0.979
	RMSE	0.627	0.824	0.395	0.394	0.691	0.825	0.888	0.789	0.109	0.099
	c^2	0.111	0.218	0.054	0.061	0.421	0.651	0.431	0.536	0.011	0.010
	PE	7.723	8.151	5.121	5.882	6.974	6.117	7.338	7.017	0.617	0.881
Saqie	R	0.917	0.909	0.941	0.936	0.933	0.932	0.923	0.921	0.991	0.978
	RMSE	0.897	0.994	0.455	0.472	0.991	0.925	0.921	0.989	0.111	0.085
	c^2	0.636	0.647	0.214	0.318	0.478	0.547	0.857	1.061	0.141	0.111
	PE	6.443	6.136	4.141	4.281	8.124	7.141	6.929	6.991	0.417	0.211
Sukkari	R	0.894	0.901	0.952	0.948	0.932	0.925	0.912	0.910	0.999	0.999
	RMSE	0.777	0.844	0.551	0.411	1.091	1.025	0.921	0.989	0.111	0.085
	c^2	0.741	0.548	0.144	0.111	0.331	0.641	0.443	0.146	0.111	0.211
	PE	4.436	6.898	4.865	7.232	6.721	5.137	5.121	4.089	0.335	0.321

	Parameters	MGAB		MHalsey		MOswin		MSmith		ANN	
		Ads.	Des.	Ads.	Des.	Ads.	Des.	Ads.	Des.	Ads.	Des.
Overall	R	0.848	0.852	0.901	0.893	0.872	0.889	0.841	0.862	0.982	0.979
	RMSE	0.874	1.241	1.001	1.013	0.981	0.955	0.984	0.900	0.071	0.077
	c^2	1.041	1.008	1.003	1.111	0.991	1.432	1.113	0.996	0.331	0.282
	PE	6.466	7.138	7.285	6.262	6.143	6.713	6.521	6.049	1.104	1.011

Figures

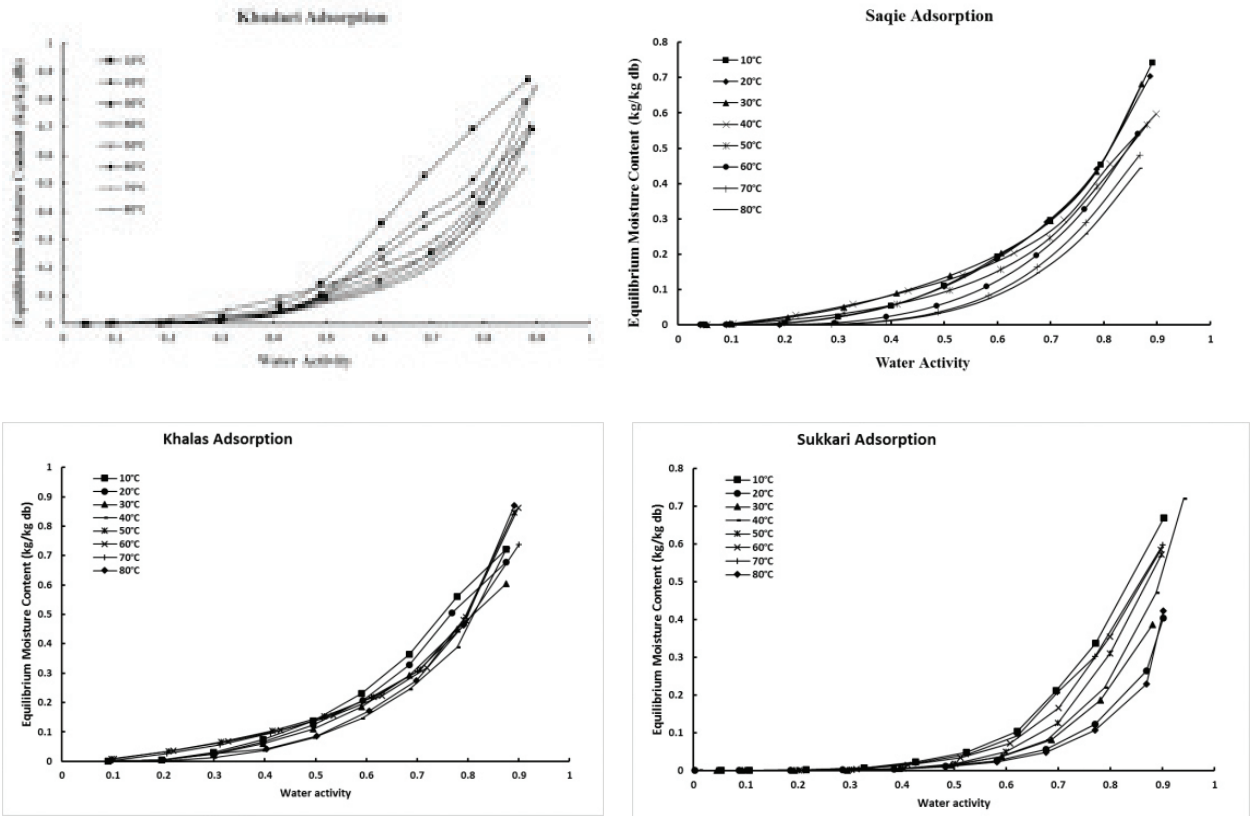

Figure (1): Dynamic vapor adsorption isotherms of date cultivars at different temperatures.

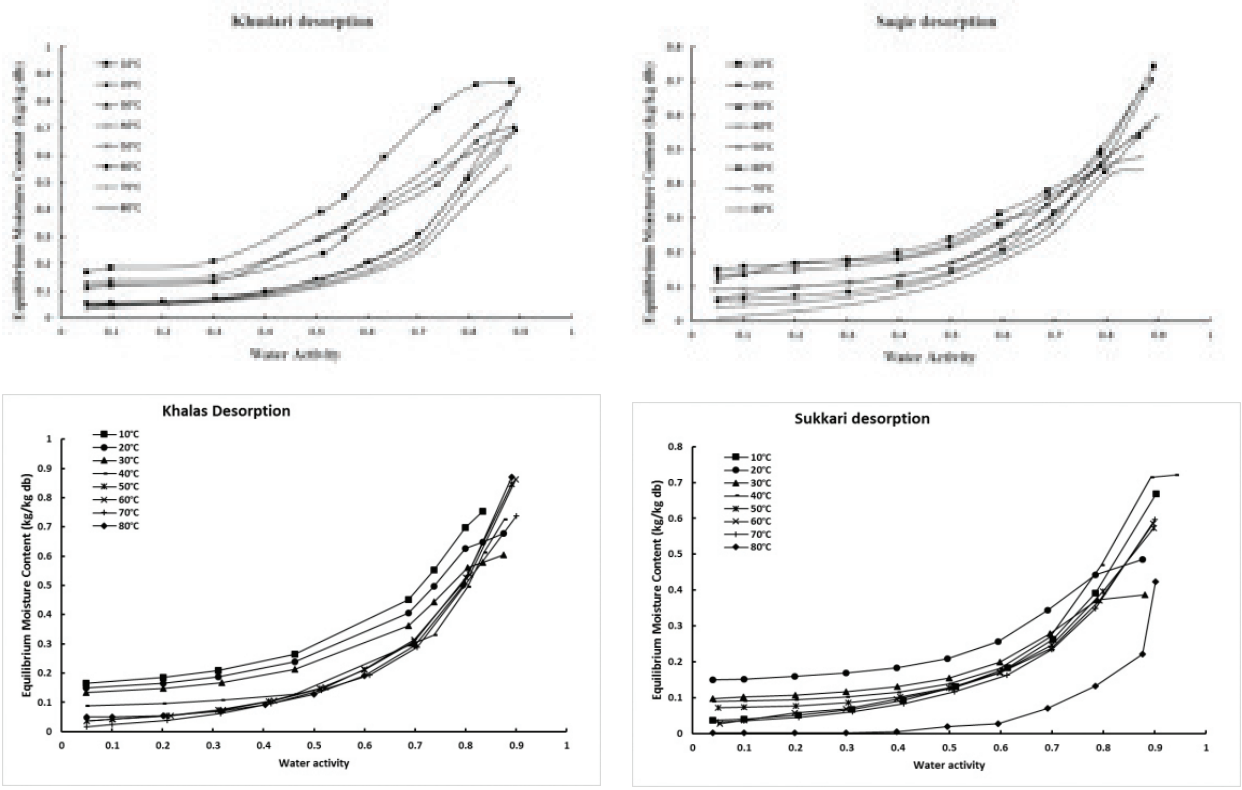

Figure (2): Dynamic vapor desorption isotherms of date cultivars at different temperatures.

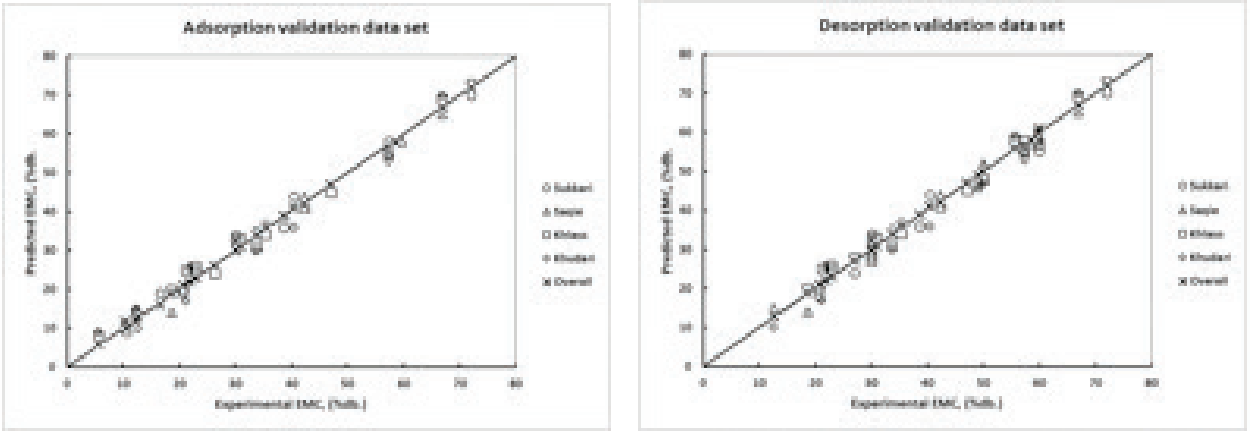

Figure (3): Adsorption- Desorption validation data set for all cultivars by selected ANN model.

Physical and mechanical properties of spray dried date palm syrup powder

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ABSTRACT

The main objective of the present study was to introduce an appropriate combination of drying conditions and date palm syrup additive for producing date powder by a spray dryer. Experiments were carried out at 150, 170, and 190 °C air temperature and 380, 420 and 460 m^3/h flow rate levels. Maltodextrin at the rate of 10, 15, 20, 25, 30 and 35% of total soluble solid of syrup was used. It was found that the higher inlet temperatures resulted lower moisture contents, bulk and tapped density, cohesion, angle of repose and internal friction angle, and higher particle size, porosity, solubility and flowability.

Keywords: Date palm, Powder, Physical properties, Syrup, Spray drier

INTRODUCTION

Date palm fruit was used as a sweetener in the ancient world and still is delectable, complex fruit syrup with a variety of uses and nutritional benefits. The date syrup is an ideal substitution for sugar in favorite baking and cooking recipes. The mineral contents including potassium, magnesium and iron, make it a great alternative to sweeten up recipes without adding sugar. Fruit powders have many benefits and economic potentials over their liquid counterparts such as reduced volume or weight, reduced packaging, easier handling and transportation, and much longer shelf life.

Chopda and Barrett (2001) produced guava juice powder using freeze-drying, spray drying and tunnel drying. It was reported that the freeze-dried product had superior quality; however the spray-dried product was stable and may be more economical. Drying of fruit juice by spray dryer has been a subject of extensive research over past decades such as dried tomato pulp by spray dryer (Chopda and Barrett, 2001; Goula and Adamopoulos, 2005), watermelon powder (Quek *et al.,* 2007), pineapple powder (Weerachet *et al.,* 2009), Nopal Mucilage powder (Leon-Martinez *et al.,* 2010), dried bayberry juice (Fang and Bhandari, 2012), raspberry juice (Anekella and Orsat, 2013), and spray drying of Nopal Mucilage (Torres, 2013).

Lack of powder production due to the juice characteristics nature is main problem that has been reported in previous research. For preventing of powder stickiness two remedies were suggested, 1) using of drying agent material and 2) using of specific equipment to facilitate the powder handling (Chegini and Ghobadian, 2007). Cano-Chauca et al. (2004) studied the effect of drying agents (Maltodextrin, Arabic gum and waxy starch) on the stickiness and solubility of Mango juice dried by a spray dryer, and found that as the cellulose concentration rises in the solution, the stickiness and solubility of the final product decrease. Goula and Adamopoulos (2010) found that the combination of Maltodextrin and use of dehumidified air could effectively produce a free-flowing orange powder. Fitzpatrick et al. (2004) conducted a research on the flowability of milk powders with different fat contents, and reported that moisture sorption had only a small effect on the wall friction of each powder with small increases at higher moistures. Moreira et al. (2009) stated that higher inlet temperatures favor the desired physical properties of the powders, decrease the moisture content and hygroscopicity, and increase powder flowability. It was also found that Cashew tree gum enhanced the powder flowability.

Considering the second rank for date fruit production in Iran (Anonymous, 2013), lack of modern date fruit processing industries, and reducing the date fruit wastes the present study was conducted to produce powder from date fruit syrup and pinpoint the effects of various spray drying

conditions (inlet air temperature and flow rate) on some of the physical and mechanical properties of produced powder.

MATRIALS AND METHODS

Materials

Date syrup with Brix of 78 was prepared from local market and used. The syrup was then diluted to 15 Brix with distilled water. To ensure of the absence of dispersive particles that cause congestion, each test sample was passed through a 60-mesh sieve. Then, syrup were heated up to 30 °C and some amount of Maltodextrin was gradually added to syrup for creating desired values of 10, 15, 20, 25, 30 and 35% total soluble solid for date syrup. The mixture was mixed by a laboratory blender for completely uniform syrup. The mixture then feed to dryer.

Test apparatus

A pilot-scale spray dryer consisted of a centrifugal atomizer wheel, cyclone air separator, a peristaltic pump, was used for the spray drying process. The main chamber was made of steel and had the inside diameter of 1.2 m and a total height of 2.4 m. The compressed air was dehumidified before supplying to the nozzle. Inlet drying air at different flow rates of 380, 420 and 460 m^3/h, heated up to 150, 170 and 190 °C after being passed through an electrical heater, and flowed concurrently with the spray through the main chamber. The produced powder samples were kept in airtight containers during experiments.

Powder properties

1. **Moisture content:** The moisture content was determined after that 5 g of powder was dried in an oven with 105°C temperature during 4 hrs (Chegini and Ghobadian 2007). The moisture content is expressed in terms of the percent in wet basis.
2. **Particle size distribution:** Screen analysis was done using a vibratory sieve shaker (Retsch GmbH and Co., Haan, Germany) with a series of seven sieves to determine the weighted mean diameter of particles as well as size distributions. The sieve sizes were 18, 35, 45, 70, 120, 270, 500 mesh, and a pan. A 50 g powder was fed on top sieve and operated at 60 Hz for 5 min (Niro, 1978d).
3. **Bulk and Tapped density:** two g of powder was poured to a 50 ml graduated cylinder, and then cylinder was shacked for 5 min. The bulk (ρ_{bulk}) and tapped (ρ_{tapped}) density was calculated by dividing the mass of the powder by the volume occupied in the cylinder (Niro, 1978a).
4. **Particle density:** Particle density ($\rho_{particle}$) of the powder sample was analyzed according to Niro (1978c). One g powder was poured into a 10 ml graduated cylinder with a glass stopper. Then 5 ml petroleum ether was added and shacked until all the powder particles suspended. Finally, all the powder particles on the cylinder wall were rinsed down with further 1 ml petroleum ether and the total volume of petroleum ether with suspended powder was read. The particle density was calculated as follows.

$$\rho_{particle} = \frac{\text{weight of powder (g)}}{\text{total volume of petroleum ether with suspended powder (ml)} - 6}$$

5. **Porosity:** Porosity (ϵ) of samples was calculated by following relationship (Jinapong *et al.,* 2008):

$$\varepsilon = \frac{(\rho_{particle} - \rho_{tapped})}{\rho_{particle}} \times 100$$

6. **Flowability and cohesiveness:** Flowability and cohesiveness of the powder were evaluated in term of Carr index (CI) (Carr, 1965) and Hausner ratio (HR) (Hausner, 1967), respectively. Both CI and HR were calculated from the bulk and tapped densities of the powder as shown below:

$CI = \frac{(\rho_{tapped} - \rho_{bulk})}{\rho_{tapped}} \times 100$	$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \frac{\rho_{tapped}}{\rho_{bulk}}$

Classification of the flowability and cohesiveness of the powders based on the CI and HR values are presented in Tables 1.

7. **Insolubility Index:** 13 g of powder was poured into a mixer jar, added in 100 ml of 24 °C distilled water and mixed for 90 seconds at 3900 rpm. Then 50 ml of sample centrifuged for 5 min at 6500 rpm, and the amount of sediment in ml was read (Niro, 1978a, 1978b).
8. **Angle of repose:** is defined as the angle between the horizontal and the slope of a heap of powder dropped from a designated elevation. About 50 g of powder poured in a funnel when bottom of funnel laid on a leveled surface. Then funnel elevated to 15 cm height. The aforementioned angle was measured using a shop protractor.
9. **Angle of internal friction:** A shear cell apparatus was made from solid plastic as shown in Fig. 1. This apparatus consisted of two cylinders with inner diameter of 3 cm and the height of 2 cm, respectively. Bottom cylinder fixed on a table and upper cylinder was movable. Both cylinders were filled with the powder and moving cylinder was connected to the universal testing machine (Santam ST-20) by a light wire cable. A cup put on the top of powder so that compressed power in cylinder. Different standard weights (100, 200, 300, 400 and 500 g) were put on the cap and the cable was pulled by Santam with a constant speed of 10 mm/min until shear force set at a nearly constant value. Relationship between the maximum values of shear

forces versus normal forces was determined by a linear regression. The values of line slope and intercept was drown out as angle of internal friction (Φ) and cohesion (C) of the powder, respectively (Coulomb equation).

10. **Powder recovery:** Spray drying yield was determined by the ratio of the total recovered product mass to the mass of initially fed mixture into the system.

Statistical analysis

Collected data were analyzed based on factorial experiment with completely randomized design. All foregoing measurements were made in triplicate. Tukey's post-test (at p=0.05) was used to determine differences among the mean values of the physical and mechanical properties of the powder samples. The SPSS software (version 16) was used for statistical analysis.

RESULTS AND DISCUSSION

Spray drying of date syrup performed two times. At first, pure date palm syrup was used without any agent materials. Results indicated that in all of the tests no powder was produced and concentrated materials adhered to the chamber wall. Then, experiments performed with adding Maltodextrin to date syrup at sex levels of total solid of date syrup (10, 15, 20, 25, 30 and 35%). Maltodextrin yielded more powder than other agent materials. About 50% yield was obtained at 35% total soluble solid of Maltodextrin. Therefore, Maltodextrin for preparing 35% of total soluble solid of date syrup was selected for further experiments.

Results depicted that inlet air temperature and airflow rate have significant effect on powder moisture content. At constant air flow rate, increasing in inlet air temperature reduced the powder moisture content. The higher drying air temperature, the higher temperature gradient at the surface of feed drops, and therefore, it expedited the heat transfer rate and moisture evaporation from the liquid drops in the drying chamber, resulting in low moisture level of dried product. The higher drying airflow rate caused a decrease in product sojourn time in the drying chamber, and less amount of moisture removal (Fig. 2). These results are consistent with those obtained for orange powder (Chegini and Ghobadian, 2007), soymilk powder (Jinapong *et al.,* 2008), Nopal Mucilage powder (Leon-Martinez *et al.,* 2010), and orange powder (Goula and Adamopoulos, 2010).

The inlet air temperature and airflow rate have significant effect on particle size. When the drying temperature is sufficiently high, moisture is quickly evaporated and the particle skin becomes dry and hard, so that the hollow particle cannot deflate when vapor condenses within the particle as it moved into cooler regions of the dryer (Fig. 3). However, when the drying temperature is low the skin remains moist for longer time, so that the hollow particle can deflate and shrivel as it cools. According to Nijdam and Langrish (2006) milk particles dried at 200 °C were spherical and smooth, while milk particles dried at 120 °C were smaller with a shrivelled appearance. Increase in drying air flow rate results more fine particles.

Inlet air temperature and airflow rate have significant effect on bulk and tapped density (Fig. 4). As evaporation rate becomes faster, more porous or fragmented structure is obtained. According to Walton (2000) there was a greater tendency for the particles to be hollow when the drying air temperature was increased. The effect of drying air flow rate on powder bulk and tapped density depends on its effect on moisture content due to the sticky nature of the product. The higher the powder moisture content, the more particles tend to stick together, leaving more interspaces between them and consequently resulting in a larger bulk volume.

The effect of drying inlet air temperature and airflow rate on powder porosity, flowability, cohesion, angle of repose and angle of internal friction depends on moisture content and particle size of powder. Results indicated that increasing the inlet air temperature, increased porosity. As particle size increases, the spaces between the particles were also increased, lead to increased porosity.

The flowability and cohesiveness of powders varied from 20.4 to 23.3 and from 1.26 to 1.30, respectively. Based on Table 1 flowability and cohesiveness of powders were intermediate and fair, respectively. The changes in flowability and cohesiveness were significant ($p \leq 0.05$). As particle size increased, the flowability and cohesively of powder was expected to decrease (Jinapong *et al.,* 2008). However, for date palm powder the smaller particles produced larger surface area per unit mass of powder, created more cohesive forces and more frictional forces to resist against the flow (Fig. 5 and 6). Moisture content has a significant effect on the flowability and cohesiveness of powder. Liquid bridges and capillary forces acting between powder particles and reduces flowability and increases the cohesiveness of powder. In addition, moisture content plasticizes the powder material, especially the water soluble constituents, results in deformation of the powder (Kim *et al.,* 2009; Moreira *et al.,* 2009).

Solubility showed a decreasing trend with the increase in inlet air temperature (Fig. 7). This was because of the effect of inlet air temperature on particle size. Increasing the drying air temperature generally produces larger size particles and increases the dissolve time of powder. Because of rapid formation of dried layer on droplet surface, no water influenced the inner of particle when dissolved in the water (Chegini and Ghobadian, 2007). More air flow rate increases the powder moisture content

and thereby decreases the powder solubility (Goula and Adamopoulos, 2005; Weerachet *et al.,* 2009).

Powder yield increased by increasing the inlet air temperature from 150 to 170 ^{0}C and the trend changed from 170 to 190 ^{0}C. The higher drying air temperatures from 150 up to 170 ^{0}C, the faster drying times and the higher powder gained. However, heating up the air more than 170 ^{0}C caused to melt of the produced powder and cemented them on the dryer wall. Increasing in air flow rate from 380 to 420 m^3/hr reduced the drying time and lead more powder yield. Whilst, increasing air flow rate from 420 to 460 removed tiny particles from the drying chamber, and resulted yield reduction.

As shows in Fig. 8 there is a linear relationship between internal friction angle (Φ) and particle size (ps). The trend of calculated cohesion (CI) and measured one (C) versus particle size followed same direction, but higher correlation was obtained between measured cohesion and particle size (Fig. 9).

Using Graph Pad 6.01 computer program the slope and intercept of CI versus C curve was compared with y=x line. No significant difference was observed between the slopes, but significant intercept implied that cohesion is overestimated by formula (C) by an error about 2.7 percent.

CONCLUSION

The results of the present study revealed that drying of date syrup without agent drying materials do not produce powder even with alteration in dryer operating parameters such as inlet air temperature and flow rate. The Maltodextrin was a suitable drying material agent to increase the dryer yield. The results of statistical analysis showed that the inlet air temperature and flow rate significantly influenced on physical and mechanical properties of date palm powder.

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Table

Table 1. Classification of powder flowability and cohesiveness

CI (%)	Flowability	HR	Cohesiveness
<15	Very good	<1.2	Low
15–20	Good	1.2–1.4	Intermediate
20–35	Fair	>1.4	High
35–45	Bad		
>45	Very bad		

Figures

Fig. 1. Apparatus for determining the shear strength of powder

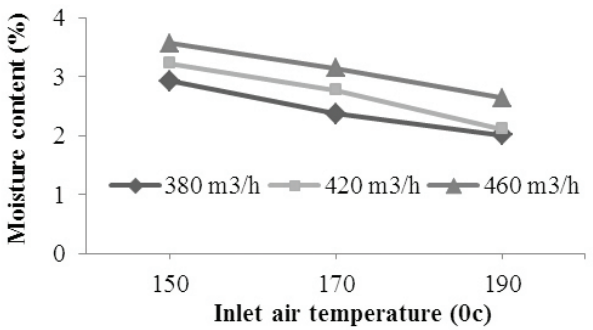

Fig. 2. The effect of inlet air temperature and air flow rate on moisture content

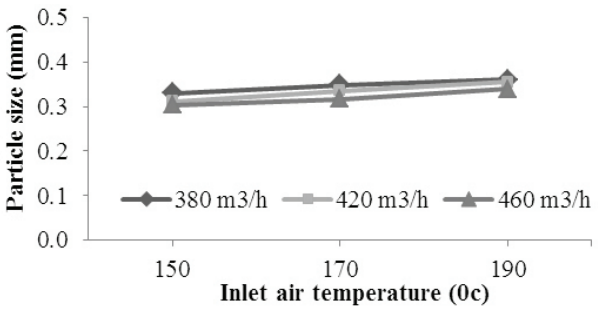

Fig. 3. The effect of inlet air temperature and air flow rate on particle size

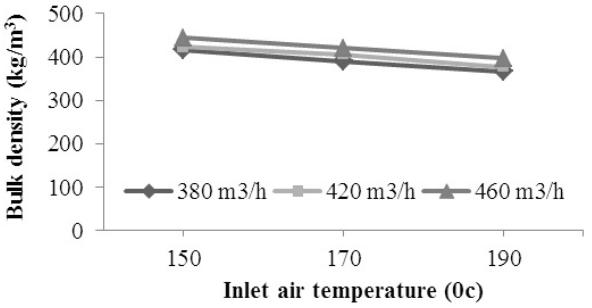

Fig. 4. The effect of inlet air temperature and air flow rate on bulk density

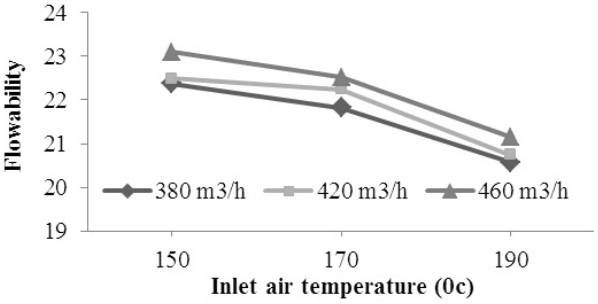

Fig. 5. The effect of inlet air temperature and air flow rate on flowability

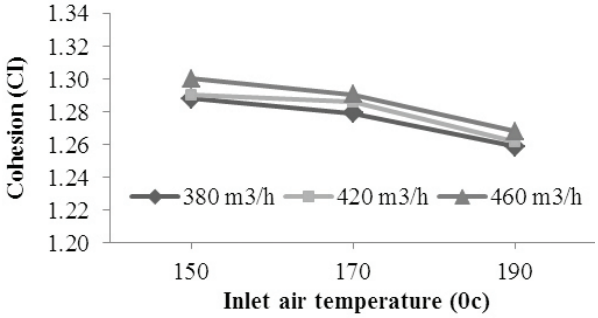

Fig. 6. The effect of inlet air temperature and air flow rate on cohesion

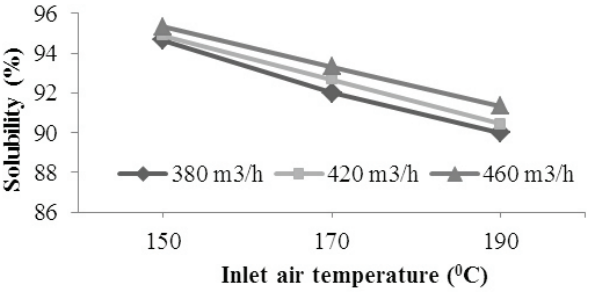

Fig. 7. The effect of inlet air temperature and air flow rate on solubility

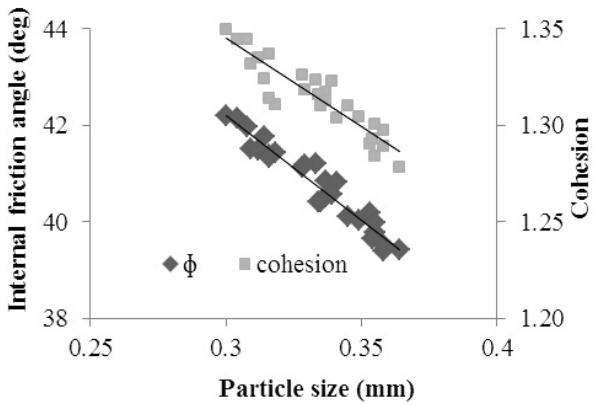

Fig. 8. Relationship between internal friction angle (Φ) and cohesion based on the particle size

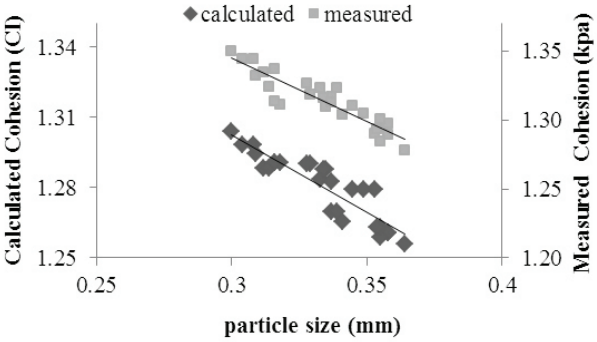

Fig. 9. Calculated and measured cohesion versus particle size

Design, development and evaluation of a pitting unit for destoning date-fruits

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ABSTRACT

Iran is the third largest producer and second larger exporter of date fruits. Pitting is one of the processes that increase the fruit economic value. In the present study a clamp-type pitting unit for date fruit was designed, developed and evaluated as a new approach. In principle each unit of the automated mechanism uses a clamp with two forks made of stainless steel, the unit is elastic in nature. Pitting unit was evaluated on mazafati cultivar at three nominal rates of pitting (30, 60 and 90/min.) and at five moisture content levels (20, 22, 24, 26 and 28 % w.b.). Analyses of the data showed that moisture content does not affect percent stone removal whereas rates of pitting had significant effects on percent stone removal. Average percent stone removal for various mc levels was 50%, similarly average percent stone removal for various pitting rates was 50%. However maximum percent stone removal of 80 % could be obtained at two combinations of pitting rate and mc level (30/min. & 26% and 60/min. & 24%). The average percent flesh loss was 2%, which is considerably low and therefore acceptable. Average change in fruit length was limited to 11% which does not seem to affect the market value of the destoned fruits. As the rate of pitting increased, the percent change in fruit diameter tends to increase too. When fruit mc increased reverse trend was noticed. Although not significant, an overall change in fruit diameter equal to 9% was noticed. this study showed that the highest pitting (80%) can be achieved when dates are pitted at pitting rate of 60/min. and mc of 26%. for this adjustment lost flesh, change in fruit height and diameter change were 2%, 11% and 9%, respectively.

Keywords: Date palm, Date fruit, Destoning, Date fruit post-harvest.

INTRODUCTION

According to the FAO statistics, the world production of dates during 1996 touched a new record of 4492000 tons (Anonymous, 2013). Iran has emerged as the world's largest producer followed by Egypt. In spite of this production level, advance research and technology for processing and packaging machinery is unfortunately not well progressed (Mikki, 1998). Automatic sorting by camera computer system has not been successful so far on commercial scales. Picking rejected dates due to various defects in the inspection line is not still visualized. Although destining machinery has been developed for some fruits, little attention has been devoted to develop destining techniques for date fruits. Destoning machines presently available are designed and fabricated mainly for cherries, apricots plums or olive, but are supplied to date packers with little modifications.

Available machines are designed and developed for limited number of varieties and therefore more research is needed to develop new machines capable of destoning in various conditions. The uniformity of fruit shape and volume which varies from one cultivar to another and proper positioning individual dates in cups are the most important problems facing development of destoning units (Chesson *et al.,* 1997).

Nourozi and Raoufat (2005) developed and evaluated a destoning unit for individual dates. The two main parts

were a singulator unit and a pitter unit. The automatic date pitter unit comprised of two parts, a cup for holding individual date fruit and a special probe to push the stone downward leaving the flesh intact. A magnetic switch operated by a timer-circuit pushes the probe into the cup in line with the centerline of the fruit placed in the cup. They evaluated the unit at various levels of fruit moisture content and impulse of the pitting probe. Results of their study showed that as the fruit mc increases, the percent flesh loss and fruit deformation increases in a significant manner. They recommend that for minimum percent weight of flesh loss and deformation, date fruit moisture content should be maintained around 25%. Oskooei-Shomali (2006) improved the performance of above destoning unit.

However, the above machine suffers from appropriate plunger movement in the fruit and poor vertical alignment of the plunger and fruit cup. Considering the drawbacks associated with the destoning units, the present study was devoted to design and development of a new destoning unit capable of moving the stone out of the fruit leaving flesh intact.

MATERIALS AND METHODS

Design and development

To realize the above objectives, a clamp-type pitting unit for date fruit was designed and manufactured (Fig 1.) In principle, each unit of the automated mechanism uses a clamp and two forks made of stainless still, the unit is elastic in nature. The assembly comprised of four main parts: chassis, power transmission, electrical circuit and finally destoning unit. The destoning unit uses a unique mechanism to pull the stone out of the fruit. A stainless steel clamp with fully flexible jaws moves forward, holds the stone and finally retracts to complete a cycle. The jaws of the clamp type unit open and close as the clamp moves forward and retracts. A platform equipped with specially designed longitudinal slots is used to actuate clamp in each pitting cycle. As the clamp retracts a cam accommodated in the guide forces the jaws open and leave the stone alone.

The power transmission assembly uses two sets of belt & pulley to decrease the electric motor speed to one-sixtieth that of the motor. A 90 W electric motor with a maximum rpm of 4000 was used to drive the unit. The speed of the unit could be adjusted to the desired level by an electrical circuit incorporating a dimmer to control output speed.

Performance evaluation

The fresh Mazafati date was prepared from local market and kept in a refrigerator at 4^{O} C. In the next stage the fruits were conditioned to adjust their moisture content to five levels of 20,22,24,26 and 28% (wb.).

Pitting unit was evaluated on Mazafati cultivar at three rates of pitting (30, 60 and 90, Nominal number of pittings/min.) and at five moisture content levels (20, 22, 24, 26 and 28 % w.b.). Each of the above rates could be established using the electrical circuit controlling motor output speed. Therefore a total of 15 treatments were considered in each replicate.

Four separate tests were conducted on samples of size 10 dates fruits (10 replicates). The tests were conducted to evaluate percent change in destoned fruit diameter, percent change in destoned fruit length, percent destoned fruits and finally percent flesh weight loss. The data were collected and analyzed and compared.

RESULTS AND DISCUSSION

Analyses of the data showed that moisture content does not affect percent stone removal whereas rates of pitting had significant effects on percent this important index. Average percent stone removal for various MC levels was 50%, similarly average percent stone removal for various pitting rates was 50% (Fig. 2). However maximum percent stone removal of 80 % could be obtained at two combinations of pitting rate and MC level (30/min. & 26% and 60/min. & 24%). Other results indicated that none of the two parameters and their interaction have significant effect on percent flesh loss during pitting operation. The average percentage of flesh loss was 2% that is considerably low and therefore acceptable (Fig. 3).

Date fruit MC levels, pitting rates and their interaction did not show to have any significant effect on percent change in date fruit length due to pitting operation. However, average change in fruit length was limited to 11% which does not seem to affect the market value of the destoned fruits (Fig. 4). As the rate of pitting increased, the percent change in fruit diameter tends to increase too. When fruit MC increased reverse trend was noticed. Although not significant, an overall change in fruit diameter equal to 9% was noticed (Fig. 5). This study showed that the maximum pitting (80%) can be achieved when dates are pitted at pitting rate of 60/min. and MC of 26%. For this adjustment lost flesh, change in fruit length and diameter change were 2%, 11% and 9%, respectively.

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Figures

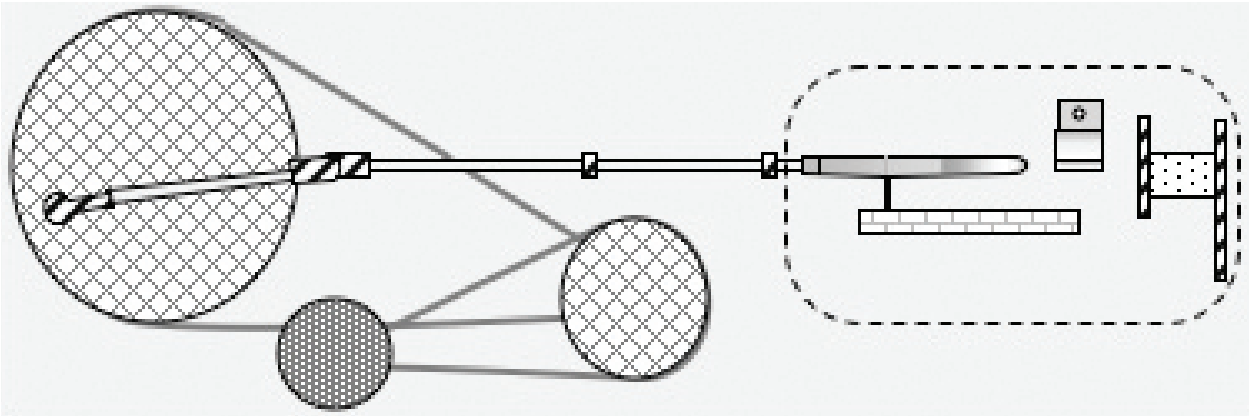

Fig. 1. Layout of the destoning unit developed in this study

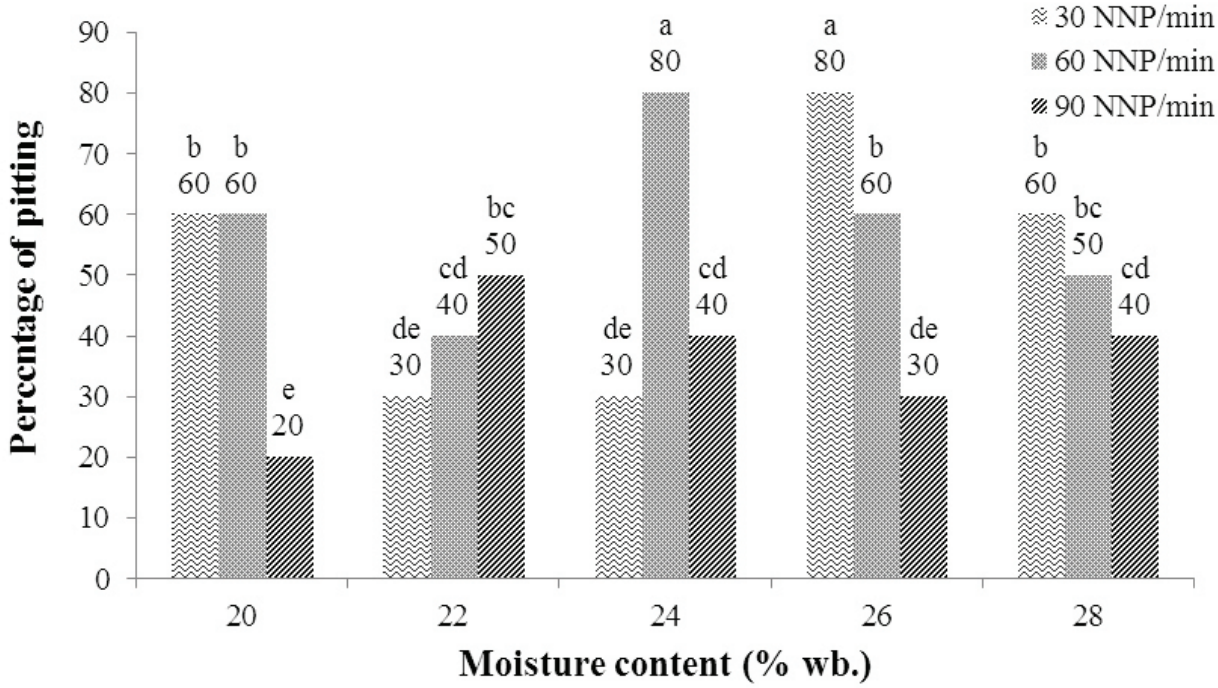

Fig. 2. Percent destoned fruit for various treatments

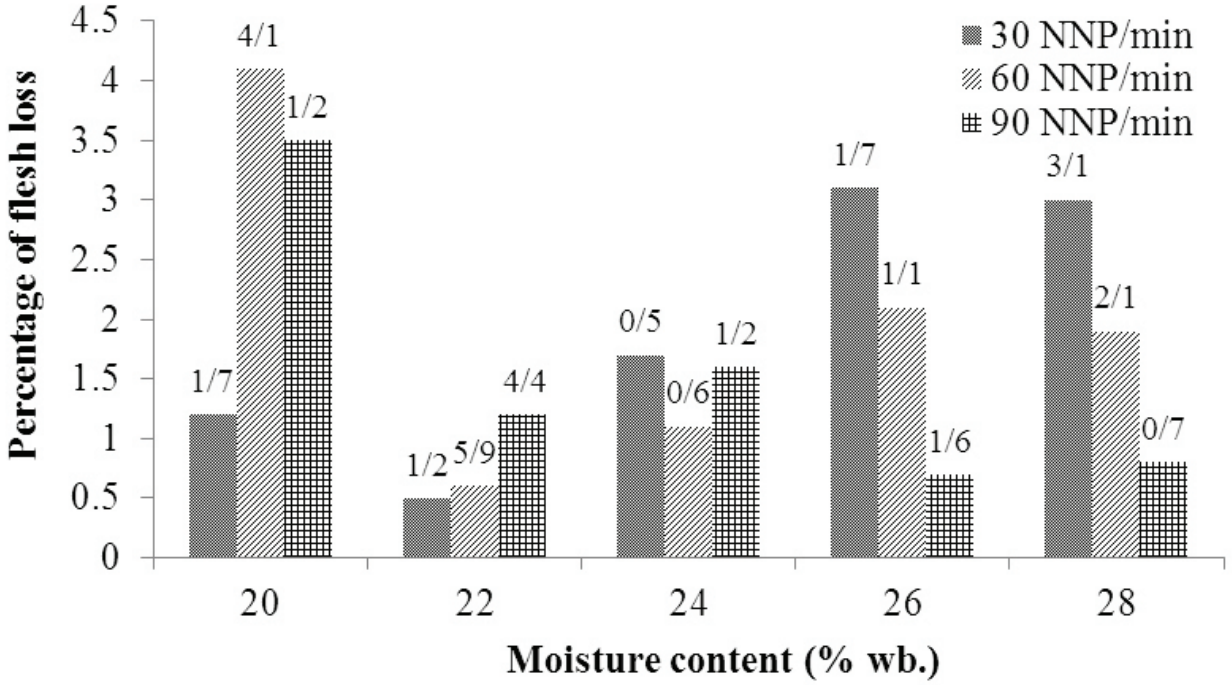

Fig. 3 . Percent flesh weight loss for various pitting rates and moisture contents

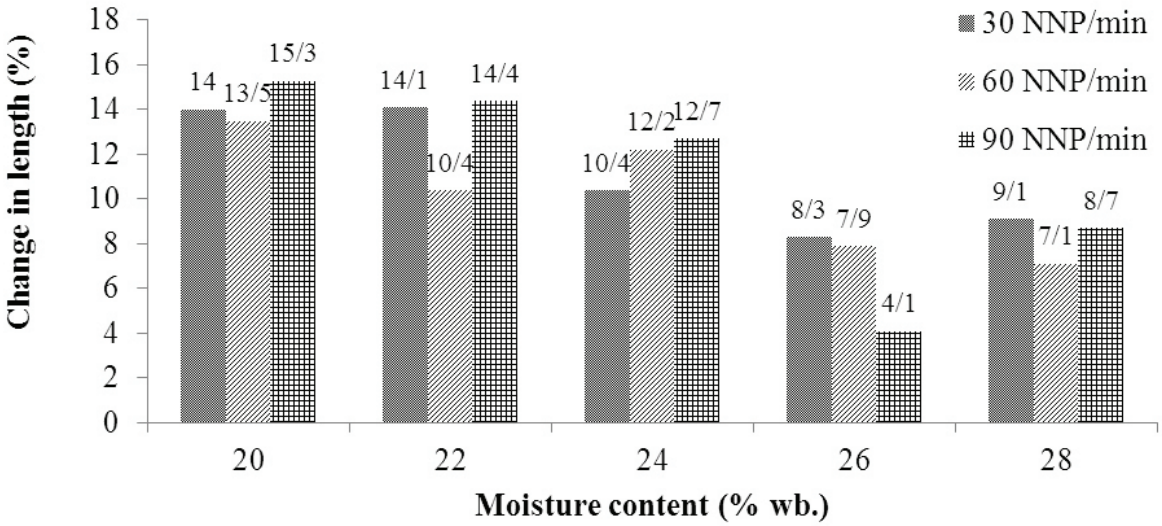

Fig. 4. Percent change in Fruit length for treatments studied

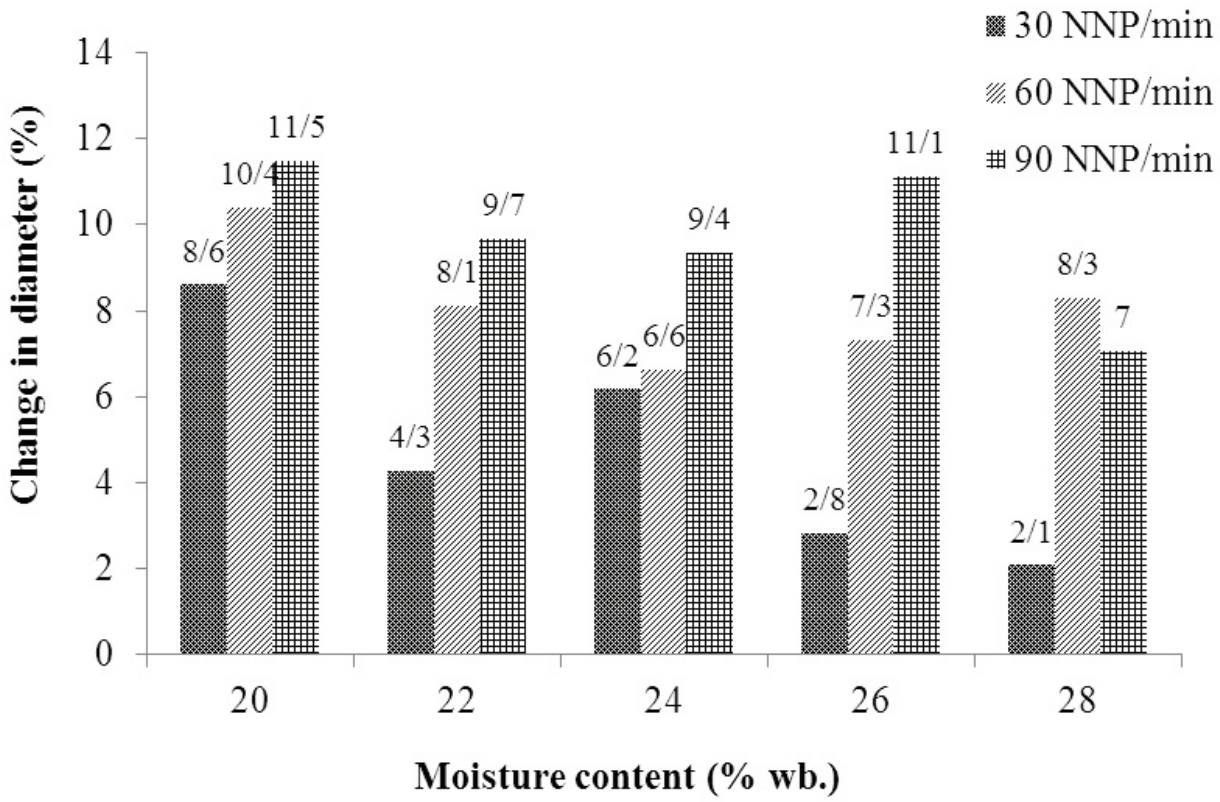

Fig. 5. Percent change in fruit diameter for treatments studied

Application of ISO 9000 quality standards and hazard analysis critical control point (HACCP) system to the date palm packaginghous for food safety and high quality

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ABSTRACT

The application of TQM by ISO 9000 quality standards and HACCP system will be essential maintaining and even expanding date palm packaginghouse export market, Pressures for quality assured products from the United States and European buyers. TQM is a broad management concept and log-term business philosophy that stresses meeting a "right first time, zero defect". Both ISO 9000 quality standards and HACCP system embody a great part of the TQM. The application of this preventive oriented approach would give the food producer better control over operation, better manufacturing practices and greater efficiencies, including reduced wastes. TQM by ISO 9000 & HACCP were introduced for the date palm line at packaginghouse for Preserved Foods , Egypt for safe and good quality foods products.

INTRODUCTION

Date processing enjoys a high economic importance in the world. Dates have nutritive values and are consumed in large quantity in all parts of the country. The main aim of the date palm packing to produce high quality and safe foods. To assure the safety of the food, establishing a system based on a continuous management including total quality management (TQM), Good Hygiene Practices (GHP) and good manufacturing practices (GMP), is essential. Therefore, Hazard Analysis and Critical Control Points system (HACCP) should be examined (Bennet *et al.,*1999). HACCP is defined as "an effective system based on GHP and GMP, for providing safe and healthy foods" (Pierson and Corlett, 1992).

HACCP is an effective system because this food safety system is designed to provide the information flow for preventive and corrective actions and can easily be established on the production lines of all kinds of foods (Unnevehr and Jensen, 1998). Safe and healthy products can be served to consumers by eliminating the safety risks after determining the critical control points by hazard analysis and establishing the necessary preventive and corrective actions (Pierson and Corlett, 1992). Whole dates are harvested and marketed at three stages of their development. The three stages are as follows: Khalal, Rutab and

Tamar:. Fruit harvested at Tamar stage is non-perishable, i.e. micro-organisms cannot grow on it, moisture uptake and its consequences, and changes in color and taste occur during storage. Most of the dates varieties are harvested after the fruit has undergone the process of ripening and drying on the palms. Fruit at the Tamar stage is ideal for marketing as "dried" dates. This fruit is used for preservation and year-round consumption and also for the production of various types of products, e.g. cakes, sauces and components of

granules or date honey. The first step to establish the HACCP system in date palm packing line should be to form the flow diagram of the production line. In this way, critical control points (CCP)can be determined on the flow diagram sample and hazard analysis can be performed. A sample flow diagram and packing operations for the process of Rutab and Tamer date palm packing line. The given sample flow diagram must be verified by the Quality Control (QC) or Quality Assurance (QA) Department of the plant.

This paper focuses on the flow diagrams based on the production line of date palm packing and hazard analysis can be performed at Date Palm Packaginghouse in Egypt.

MATERIALS AND METHODS

Development and implementation of HACCP system.

The steps used to develop and implement the HACCP system as appropriate to particular industry under consideration as described by Stevenson & Bernard (1999) as follows.

Prerequisite Programs.

Good Manufacturing Practices (GMP) Good and Hygiene Practices (GHP). Basic environmental and operating condition as described in the Wallace and Williams (2001).

Application of HACCP seven principles and FAO (2001) recommended 12 task in development of HACCP plan for date palm packing processing line based on (Figure 1)

RESULTS AND DISCUSSION

All the stages of implementation were followed stage by stage, all the procedures necessary of control and checking were established to check and confirm if HACCP / ISO 9000 system is implemented in accordance with the principles of the codex and standard ISO 9000/2005. The analysis of the risks was carried out to identify the hazards which can occur in the cycle of production, the preventive measures were established, CCPs and OPRP was determined and posted at the factory, the critical limits for each CCP were defined and validated. A monitoring system is established to be ensured if the critical limits are respected and OPRP are mastered. The recordings relating to this monitoring are held up to date. Procedures of checking were established to confirm if plans HACCP/ISO 9000 are effective (internal audits). Thus documentation concerning all the processes, the procedures, measurements and the recordings were appropriate with the nature and the size of the company.

Implementation of HACCP plans and Operational Prerequisite programs (Figure 1).

1. HACCP team.

A multidisciplinary team was composed of seven persons possessing different skills related to quality assurance, production, engineering, microbiology and so on. Members of this team have been trained very thoroughly on the HACCP and ISO 9000.

2. Product description.

Whole dates are harvested and marketed at three stages of their development.

1. Khalal: Physiological mature, hard and crisp, moisture content: 50 - 85 %, bright yellow or red in color, perishable;
2. Rutab: Partially browned, reduced moisture content (30 - 45 %), fibers softened, perishable;
3. Tamar: Color from amber to dark brown, moisture content further reduced (below 25 % down to 10% and less), texture from soft pliable to firm to hard, protected from insects it can be kept without special precautions over longer periods.

3. Identify Intended Use (Task3)

The normal expected use of the food was described. With regards to possible acceptable risk level for a food safety hazard it has to be stated for which group of population the food is intended (Untermann, 1999). The intended use need to be stated or informed whether the food need to be prepared prior consumption. Besides that sensitive consumers too need to alert which adequate information on allergenic ingredients if it were used to prepare the product.

4. Flow diagram.

Flow diagrams have been prepared taking into account all aspects of the process in the scope of the HACCP system. The flow diagrams were checked on site by the HACCP team (Figures. 2and 3).

5. Onsite confirmation and verification of process flow (Task 5)

The HACCP team shall perform onsite verification on the accuracy and completeness of the flow diagram. Besides that the team also was trained to check the conformity of flow diagram is correct for any shift pattern that normally takes place in processing plant (Slatter, 2003). The onsite assessment normally involves participation of respective responsible personnel to explain the processing nature and the operation procedure during assessment (Tables 1 and 2) . During the assessment, any additional documentation required for on-site review was examined (Motarjemi, 2000).

Each step was checked and to ensure that all relevant information regarding potential hazards to the process and products are identified. If any modification required, it were amended immediately and documented. After the five preliminary tasks have been completed, the seven principles of HACCP are applied to construct the HACCP plan (Corlett, 1998).

6. Hazard Analysis on the Production Line

After constituting the flow diagram to determine the critical control points (CCP), hazard analysis can be performed (Scott and Moberg 1995). Possible risks that may occur during the production must be taken into account and necessary preventive actions must be determined.

7. Critical Control Points (CCP) on the Production Line

After hazard analysis, determined risks should be considered by decision tree if they are critical control points or not. Then, factors that constitute the hazard should be determined. Parameters used during monitoring critical control points, critical limits, preventive and corrective actions, and production and operation instructions and responsibilities of the staff should be well defined (Codex Alimentarius Commission, 1993). To monitor these activities, necessary forms and records should be kept as an archive for internal and external audits (Annon, 1998). Inspection and storage of fruits date (raw materials); sorting; cleaning; washing; drying; transporting to the packinghouse, and serving/distributing the markets, are the critical control points in the packaging lines (Tables 3 and 4).

7.1 Harvesting the fruits date

Harvesting the fruits date entails the use of experienced workers, or investment in aluminum ladders, in attaching ladders to the palms permanently or in purchasing mechanical appliance to lift workers to the top of the palm. Rain can cause damage to the fruit and impair its quality due to rotting, fermentation and insect infestation. On the other hand, the fruit purchases raw materials from several contractors. The production requires a stock monitoring program and raw materials should be purchased as closer as possible to the production time (Bryan, 1992)

Fruits date raw materials that have microbiological loads over critical limits must be avoided to ensure food safety and quality. Toxins synthesized by microorganisms; and pesticides, chemical residues and foreign materials found in these raw materials are also potential risks for consumer health.

7.1.1 Control

The fruits date raw materials must therefore be protected against rain with the help of wax-covered paper or nylon sleeves. Harvesting must be faultless and clean, since it significantly affects the rest of the process (packing and marketing). Harvesting the fruit straight into containers suitable for transport to the packinghouse prevents the infection of the fruit by the soil and sand under the palm and ensures that the fruit arrives in good condition, and that it is not crushed.

Fruits date raw materials should be purchased in accordance with the “Raw Material Acceptance Criteria” determined by QC/QA Department. QC/QA staff members have to reject unsuitable raw materials. Microbiological, physical and chemical characteristics that raw materials must have corresponding and critical limits should be determined in “Raw Material Acceptance Criteria”. Contractor having quality certificates like ISO Quality Assurance Systems and HACCP system should be preferred. QC/QA staff member have to control the expiration date of the packaged foods. Ripped, pierced, damaged and abnormal shaped packages have to be refused.

7.1.2. Monitoring and Keeping Records

During monitoring the inspection and acceptance of fruits raw materials, responsibilities of the department staff and controllers, inspection methods and instructions have to be clearly brought up for consideration “Raw Material Control Procedures”. QC/QA staff members should keep the acceptance records and fill the necessary forms .

7.2 Storage of fruits date Raw Materials In the packinghouse

In the packinghouse there are a number of processes, designed to improve or maintain fruit quality. These processes are: fumigation, washing, storage, refrigeration, hydration, dehydration and curing. Fumigation must not be carried out when the fruit is fresh, harvested at the Khalal stage, or when stored under deep refrigeration. The substance most frequently used for fumigation is methyl bromide (CH3 Br), which makes most of the insects come out before they are killed by the gas. The concentration of the gas is 30 ppm, i.e. 30 g methyl bromide in 1 m3 of air. The time recommended for fumigation is 12 - 24 hours. The temperature must be above 16^{o}C. It is important for the air to swirl within the fumigation installation, in order for it to spread uniformly within the chamber. In the storehouses the produce must be protected from recontamination by pests (insects and rodents). The surfaces and packages must be well made in order to withstand being loaded, shaken on the way and unloaded. Today, the temperature commonly used for long-term preservation of dates of several varieties is - 18^{o}C (0^{o}F). This temperature decreases possible water loss and also decreases the sugar crystallization and skin separation phenomena.

Storage under conditions of 26 % humidity or higher requires a temperature of o^{o}C enabling a storage period of 6 - 8 months; the storage period can be more than 1 -year if humidity is less than 26 %; if humidity is

less than 20 %, dates can be stored at 25ºC for up to 1-year; and high sugar content coupled to high humidity tends to aggravate the situation of fruit going bad.

7.2.1. Possible Risks

Because of insufficient and improper storage conditions, rapid microbial growth can be seen. Cross contamination of the pathogen microorganisms from storage places to production area is another important hazard (Bryan, 1992).

7.2.2. Control

"Storage of fruits date Raw Materials" should be determined by QC/QA department for proper storing.

7.2.3. Monitoring and Keeping Records

QC/QA staff members are responsible for proper storing conditions. Temperatures and relative humilities of the storage places should be monitored by thermocouples and hygrometers continuously. Temperatures and relative humilities of the storage places, and changes in these parameters should be recorded; when necessary, these parameters should be reset. Sanitary and hygienic conditions of the stores are very significant to avoid the contamination. In addition, hygienic barriers might be used and stores should be cleaned and sanitized periodically, and records mentioned in "Storage of fruits date Raw Materials", should be kept for archive and audits.

7.3 Washing Fruits

Dates exposed to various types of contamination of physical, chemical or/and microbiological nature. Physical factors: Sand and soil - both as a result of sand storms in many regions where dates are grown, and soil sticking to fruit lying on the ground. Chemical factors: These are especially remnants of pesticides, some of which can be removed by washing. Microbiological factors: External cleaning of the fruit by washing removes some of the microbiological pollution, also excretions of birds, which may spoil the fruit.

Clean water must be used and care taken that all the fruit is washed. Other methods exist, such as damp towelling attached to sloping mechanical shakers. While the fruit is still hanging, it can be cleaned by water spray, accompanied by the use of fine swivelling brushes, but they must be dried before being packed. When the fruit is packed immediately after washing, it is important to dry it in drying cubicles or by means of large fans.

Washing and rinsing periods, chlorine concentrations, temperatures and pressures of washing and rinsing water should be adequate to remove dirtiness and to decrease the microbial load.

7.3. 1. Possible Risks

An inadequate washing program causes non-removal of physical, chemical and microbiological hazards present in natural flora of fruits. Potable water should be used for washing process, otherwise, fruits can be contaminated by unclean water. An effective rinsing is very crucial to remove chlorine from fruits.

7.3. 2. Control

A detailed "Raw Material Washing Program" should be prepared by QC/QA department for considering parameters such as the concentration of chlorine, washing and rinsing period, pressure and temperature of water according to the type of the raw material.

Generally, 50-125 ppm active chlorine is adequate for eliminating the microbial risks of the fruits and vegetables (Aran *et al.,* 1987). For very dirty raw materials 1-5 ppm active chlorine should be added to the final rinsing water (Aran *et al.,* 1987). To avoid the contamination from water used for washing, water analysis (chemical and microbiological) should be performed by authorized laboratories periodically.

7.3. 2. Monitoring and Keeping Records

QC/QA department is responsible for an effective washing and rinsing. "Raw Material Washing Program" should be applied completely. Water analysis reports should be kept for archive and audits.

7.4 Washing and Rinsing the Equipment

Dirty equipments are one of the main sources of physical and microbiological contaminations. Therefore, an effective equipment cleaning program should be applied (Bryan, 1992).

7.4 1. Possible Risks

Hazards at this step are closely related to the effectiveness of the washing program. If the washing program is inadequate, it is impossible to remove physical, chemical or microbiological hazards. On the other hand, inadequate rinsing causes non-removal of detergent, chlorine and caustic from equipment.

7.4. 2. Monitoring and Keeping Records

Concentration of active chlorine, caustic or detergent used, washing and rinsing periods, temperatures and pressures of washing and rinsing water should be clearly determined. General cleaning of equipment used in production should be periodically done by caustic solutions. Because has toxic effect on health, it should be checked whether it was removed completely from the equipment or not after rinsing. Presence of caustic on the equipment can be detected by a test in which the colorless phenol phytalein turns into purple when dropped on the surfaces if caustic is still there (Troller, 1993).

7.5 Metal Detectors

7.5 1. Possible Risks

It is possible that metal particles can contaminate the fruits during production. These metal particles may come from raw materials that are not properly handled during harvest and may cause physical hazards.

7.5. 2 Control

Control is done by metal detectors.

7.5 3. Monitoring and Keeping Records

QC/QA department staff should constitute a detailed "Metal Detector Manual". In this manual, dimensions of metal particles that metal detector should determine must be given (Mortimore, 1994). QC/QA staff member, responsible for this operation, should periodically check the detector by test and should calibrate it frequently.

7.6 Distributing

7.6 1. Possible Risks

Because of unsuitable distributing conditions, microbiological growth and spoilage of meal may occur.

7.6 2. Control

Distributing of fruits should be performed according to "Distributing Procedure" stated by QC/QA department. During transportation, temperature of the fruits should be -20ºC for Rutab and 5ºC for Tumer. To ensure that, fruits should be distributed in boxes (Bryan, 1992).

7.6 3. Monitoring and Keeping Records

Final product should be placed into boxes and distributed as soon as possible after production. Lids of the boxes should be closed tightly and checked. Also refrigerator conditions must be ensured for fruits products. Loading of the boxes into the cars should be done according to the distributing route.

8. Keeping Records and Verifying

QC/QA department should ensure to avoid the potential hazards in all steps of the process by stating preventive and corrective actions. Effectiveness of the HACCP system can be stated by verifying. All the activities taken place in HACCP system should be kept as records and forms, and archived for periodic internal and external audits. Audits are performed by Production Management Department and government officials dealing with food safety (Annon., 1998).

CONCLUSION

HACCP should be considered as a system based on Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP). GMP and GHP applications include building, environment arrangements and personnel hygiene and behaviors. Sanitary and hygienic conditions of the plant can be improved.

For serving high quality and safe products to the consumers, inspecting the raw materials purchasing, storing the raw materials at proper conditions, using well cleaned equipments in all steps of the fruits date packing process, according to receipts stated by department while storage are determined as critical points. Distribution also should be performed according to distributing instructions.

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Tables

Table (1): Green or ripe dates (Rutab) Packing Operations

Production steps	Description
1	Fruit Harvesting
2	Fruit reception & Weighing
3	Grading & Selecting
4	Storage at 5°C / fumigation
8	Dry sorting
9	Water soaking
10	Fruit spray washing
11	Fruit sorted on conveyor
12	Fruit spray washing
13	Fruit Freezing
14	Fruit packed into either: *Bulk Pack (in cardboard boxes) 5 kg **Retail pack (box, placed in two layers, separated by cellophane, weighing 220 g - 250 g.)
15	Bags or boxes transfer to labeling department
16	Bags or boxes moved on conveyor to the labeler
17 17 (i) 17 (ii)	Bags or boxes labeled Glue Label
18	Coding Fruit packed with ink jet printing with date of production and expiry date
19	Cardboard tray manually
20	Bags placed on tray by hand
21	Trays is labeled
22	Trays is shrink wrapped
23	Fruit frozen and storage at -20°C/ Humidity 70%
24	Transportation for distribution
25	Distribution

Green or ripe dates (Rutab): Partially browned, reduced moisture content (30 - 45 %), fibres softened, perishable;

Table (2): Tamer Packing Operations

Production steps	Description
1	Fruit Harvesting
2	Fruit reception & Weighing
3	Grading & Selecting
4	Storage at 5°C / fumigation
8	Dry sorting
9	Water soaking
10	Fruit spray washing
11	Fruit sorted on conveyor
12	Fruit spray washing
13	Fruit Drying
14	sorting second time
15	Production Dates lines:
15 (i)	Bulk Packing line
15 (ii)	Pitting/Pressing Line
15 (iii)	Thermo pack Line)
15 (iv)	Date juice (Dibs)
16	Fruit packed into either: 50g, 100g, 200g and 500g in PET polyethylene bags or varying sizes boxes (1kg, 2kg, 3kg, 5kg and 10kg)
17	Bags or boxes transfer to labeling department
18	Bags or boxes moved on conveyor to the labeler
19 19 (i) 19 (ii)	Bags or boxes labeled Glue Label
20	Coding Fruit packed with ink jet printing with date of production and expiry date
21	Cardboard tray manually
22	Bags placed on tray by hand
23	Trays is labeled
24	Trays is shrink wrapped
25	Fruit storage at 5°C
26	Transportation for distribution
27	Distribution

Tamar: Color from amber to dark brown, moisture content further reduced (below 25 % down to 10% and less), texture from soft pliable to firm to hard, protected from insects it can be kept without special precautions over longer periods.

Table 3. Rutab date fruits HACCP analysis chart

Step	Activity	CCP No.	Hazard Nature	Preventative Measure	Procedure	Corrective Measure	Responsibility
1.	Rutab Date Fruits Harvesting. Weighing		Loss of weight Equipme nt Defect	Check balance Calibration Periodical maintenance	?? Inform Maslaha Maintain	Adjust Calibrate Maintain	Maintenance?? Maslahat Maintenance
1.1	Sample for acceptance		Incorrect sampling by Date Fruits damage	Improper sampling Effective supplier assurance	QC to check	Train committee	QC1
1.2	Sample for independents laboratory	1	Pesticides/fungicides Heavy metals, Patulin	Effective supplier assurance	QC to take Sample per supplier	Change Supplier Inform purchasing	Purchasing Dept.??
1.3	Sample for internal QC laboratory	1	Staph. MC4 *Bacillus cerius*	Effective supplier assurance	QC to take sample per supplier	Inform supplier	Purchasing Dept.??
2	Grading & Selecting		Date Fruits damage	Good Manufacturing Practice	PR2 visual check	Inform labour supervisor	
2.1	Storage at 5°C / fumigation						
2.2	Dry sorting		Improper sorting by labors Fruit damage Foreign bodies	Good Manufacturing Practice Effective supplier assurance	visual check ??	Inform labour supervisor ??	
3	Water soaking						
3.1	Raw water	2	Portability of water Pesticides, Heavy metals, Pathogenic presence	Chemical analysis Chemical analysis Chemical analysis Microbial analysis	QC sample to QC lab QC1 sample to outside lab QC sample to QC lab	Contact water supply company for discussions	

Step	Activity	CCP No.	Hazard Nature	Preventative Measure	Procedure	Corrective Measure	Responsibility
3.2	Soaking process		Microbial contamination of water Foreign bodies	Check water in basin Effective soaking	to check to check	adjust drain rate adjust/repair air flow	Line supervisor Line supervisor
4	Spray washing		Foreign bodies	check soaking process (nozzles, conveyor)	to check to check	clean nozzles adjust conveyor rate	Line supervisor Line supervisor
5	Conveyor sorting		Improper sorting by labors Fruit damage Foreign bodies	Good Manufacturing Practice	to check	Inform labour supervisor	
☒ 6.	Spray washing		Foreign bodies	check soaking process (nozzles, conveyor)	to check to check	clean nozzles adjust conveyor rate	Line supervisor Line supervisor
☒ 7.	Dry sorting		Improper sorting by labors Fruit damage Foreign bodies	Good Manufacturing Practice Effective supplier assurance	visual check ??	Inform labour supervisor ??	
☒ 8	Freezing						
8.1	Screening in finisher		Broken seeds and fibers Microbial Contamination	Check screen integrity Check screen clean	to check	inform maintenance inform sanitation	
8.2	cooling in chiller		Outgrowth of spore due to slow cooling Microbial contamination	Effective temperature control Effective cooling time ????? Check chiller clean Check leaks in tubes Take sample of pulp for analysis	to record temperature to record time to check sanitation to check sanitation	adjust temperature	re-cool or dispose re-cool or dispose Inform sanitation

Step	Activity	CCP No.	Hazard Nature	Preventative Measure	Procedure	Corrective Measure	Responsibility
9	packing		Microbial Fermentations Plasticize contamination	Check cleaning of packages Check type of plastic used		Fast freezing and chick program Inform supplier	sanitation
9.1	Bulk Pack (in cardboard boxes) 5 kg		AS (14 (3)				
9.2	Retail pack (box, placed in two layers, separated by cellophane, weighing 220 g - 250 g.)		AS (14 (3)				
9.3	50kg plastic drum		AS (14 (3)				
10	Rutab Date freezing & storage	3	Spoilage or Fermentation of Rutab Poor Temperature cantonal Ineffective freezing process change of organoleptic properties	Microbiological analysis check thermometer check freezing cycles physical measurements		Foot freezing Adjust temp at - 18 C repair or replace reject or re use	
11	Boxes on conveyor		Broken bags / boxes Loss of production Conveyor defect	quick removal of broken bags / boxes effective boxes can tool check of speed conveyor	as above	Replace detector two every hour increase light source inform maintenance	

Step	Activity	CCP No.	Hazard Nature	Preventative Measure	Procedure	Corrective Measure	Responsibility
12	Visual inspection for fill level and bags / boxes defects	4	bags / boxes damage Loss of production glass and other foreign boding low or high level of filling	quick removal of damage bags / boxes) effective bottles *** GMP check of filler	as above	Replace detector two every hour increase light source inform maintenance	
13	Labelle tray		wrong label, wrong design poor print quality label cooked wrong size label not glue on bottles, label missing	flow specification	as above	inform supplier	as above
14	shrink wrap		wrong size wrong gauge inconsistent gauge	check roller size, cleaning check gauge check film type check tunnel temp.	as above	inform supplier calibrate gauge inform supplier adjust temp. calibrate thermometer	
15	Transportation to warehouse					check transportation system	storage Dept.
15.1	Product of warehouse		loss of production	Audit for first infest autorotation		Improvement of storage conditions	storage Dept.
16	Transportation for distributor		Exposure of predict to adverse environmental condition like dust, rain, direct sunlight Loss of production	cover with suitable cover for production from rain and direct sunlight			
17	Distribution		Loss of production	Record customer / delivery details		following specification of distribution	selling Dept.

Figures

Five Preliminary Steps

(Task 1) HACCP team Assembling

(Task 2) Product Description

(Task 3) Identification of products intended use

(Task 4) Construction of flow diagram

(Task 5) Onsite verification and confirmation of flow diagram

Seven HACCP Principles

(Task 6) Conduct a Hazard Analysis

(Task 7) Determine the Critical Control Points (CCPs)

(Task 8) Establish Critical Limits

(Task 9) Establish CCP monitoring procedures

(Task 10) Establish corrective action

(Task 11) Establish Verification Procedures

(Task 12) Establish Documentation and Record Keeping

Figure 1: 12 task sequence steps for HACCP application

Fruit harvesting
â
Fruit reception & Weighing
â
Grading & Selecting
â
Storage at 5oC / fumigation
â
Dry sorting
â
Water soaking
â
spray washing
â
Sorted on conveyor
â
Spray washing
â
Freezing
â
packing
â
Coding & Labeling
â
frozen and storage at -20oC/ Humidity 70%
â
Transportation
â
Distribution

Figure 2. The main procedures to Green or ripe dates (Rutab) Packing

Fruit harvesting
â
Fruit reception & Weighing
â
Grading & Selecting
â
Storage at 5oC / fumigation
â
Dry sorting
â
Water soaking
â
spray washing
â
Sorted on conveyor
ß
Spray washing
â
Drying
â
Sorting
â

Vacuum Packing
â
In PET polyethylene bags 50g, 100g, 200g Boxes (1kg, 2kg, 3kg, 5kg and 10kg)
â
Coding & Labeling
â
Shrink wrapped
â
Storage at 5oC
â
Transportation
â
Distribution

Figure 3. The main procedures to Tamar dates Packing

Extraction and purification peroxidase enzyme from Zahdi date seeds

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ABSTRACT

Peroxidase enzyme was prepared from germinated Zahdi date seeds (nuclei) which passed to young radicals as a source for the production of peroxidase converted to acetone powder instead of extraction from palm leaves or other plant sources. Method of extraction characterized simple and low-cost.

The results obtained showed that roots of seedlings contain a twice enzyme activity effectiveness than peroxidase of palm leaves. Soluble peroxidase activity was 296 400 units / g in acetone powder of roots, while it was 156 750 units / g in acetone powder of leaves. As well as access of enzyme peroxidase in any season in the year.

Soluble peroxide purified by several steps, the number of purification steps was 7.52 time and enzymatic recovery 27.6 at the end of gel filtration stage, results of purification indicated that there is a high affinity of purified enzyme towards substrate consisting of orthodainzdin and hydrogen peroxide.

INTRODUCTION

Characterized enzyme peroxidase it spread wide in nature, the diversity of forms in the types of the plant kingdom and within the same species and characterized his specialty wide toward the substrates and thus multiple types of metabolic reactions and processes physiological involving the growth and development of various plants (Kelly & Latzko 1979), also became the peroxidase in many plants from reliable indicator of genetic discrimination in the detection of stress in plants as well as follow-up and study the mechanism of the effect of pathogens on plant families (Lojkowska & Holuhowska 1992; Hassni, et. al, 2004). Enzymes peroxidases became important industrially and medically, peroxidase were gained (peroxidase hours radish wild HRP) great economic importance through its uses in the number of diagnostic and analytical varied because of his qualities kinetic and physical and chemical suitable for such applications and perhaps the most important number is the number of ELISA (Enzyme -Linked Immuno Sorbent Assay) in labeling antibodies or antigens in the immune reactions by attaching these enzymes on solid surfaces as it is the specifications that qualify for that (Avrameas & Guibert, 1972) one of these properties the qualities high affinity toward the material basis, ease of detection effectiveness configures outputs of color do not need a process to measure the steps separating from substrates, high persistence during storage, low costs prepared and purified (Tijseen, 1985) as well as its importance in the analysis of the stigma blot assays and in pigmentation tissue (Belonogova & Mekler, 1996), it is also used in biochemical analysis to estimate the hydrogen peroxide generated by some systems, such as the oxidation of glucose, amino acids and cholesterol .... etc. through the electrode specialist for enzyme or with other enzymes (Lin, et. Al, 1990), also reflected the importance of these enzymes in the processing of fruits and vegetables by evaluating the content extracts of food stuff from antioxidants such as ascorbic acid, phenols, flavonoids and tannins, which working on multiple modifications during the manufacturing process and storage (Cano, et.al, 1996).

As well as the economic importance of the palm and its products (dates), the Palm side-off products have many uses, moreover the issue of the exploitation of the nuclei of the surplus dates for the need to produce materials with economic value be of great benefit and add value to palm and their products.

So it is appropriate to identify the nature of the peroxidase enzyme in roots and palm seeds and determine the appropriate conditions for the extraction and identification of suitability for analytical uses, where it is known that peroxidase of the palm roots was used in assessing activity of the glucose oxidase enzyme GOD, also used to measure the concentration of blood glucose and some other sugars in food samples with the participation glucose oxidase enzyme, also used to investigate the efficiency of the processing of canned fruits and vegetables, juices, by assessing the antioxidants content (Dalali,1983).

Hydrogen peroxide works as a recipient while working AII2 as donor and oxygen was not the result of the reaction, and that enzyme works includes four main types of activities: Peroxidatic, Oxidatic, Catalaitic and addition a hydroxyl group (Hydroxylation), peroxidase works on the oxidation of many phenolic compounds by its Peroxidatic activity like Guaicol, Res orcinol and Aniline ... etc. (Whitaker, 1972) when these compounds act as substrate. The Oxidatic activity were founded when the substrate occurred, such as acid dihydroxy fumaric or ascorbic acid or indole acetic acid (1AA) with oxygen (Helser, 2008), can also peroxidase added a hydroxyl group to the number of aromatic molecules such as tyrosine and phenylalanine and benzoic acid and salicylic acid (Buhler & Mason, 1961).

Peroxidase and its purified isomers from plant sources consist colorless glycoprotein which is associated with Ferriporphyrin each one molecule per in peroxidase owns one group of Ferriporphyrin 111 which distinguishes this group (prosthetic group) being strongly linked with protein part in the enzyme (Gaspar, et.al, 1982). Focused the vast majority of studies on the production of peroxidase from horseradish roots and wild rape and fig juicer fig (Paul, 1986), while not available for detailed studies of peroxidase palm.

The research aims to exploitation seeds Zahdi date remaining as by-products from factories of syrup and honey dates by germinated seeds then extract peroxidase of the seed roots and purified, due to the abundance and activity and possibility of getting it in any season of the year compared with peroxidase extracted from other plant sources.

MATERIALS AND METHODS

Materials

Seeds (nuclei's) of Zahdi date, sodium hydroxide solution (10 molar), large bowl covered with large size filter paper, then moisturizer with water, sought to spread 60-75 seed / 30 cm (diameter dish),sodium acetate buffer solution (1molar) with pH 6 .

Solution potassium phosphate buffer (0.005 molar), O - Dinazidine concentration solution (23 mM) and glucose oxidase enzyme solution.

Methods

- Create a seedling : Zahdi date seeds was germinated after being washed with distilled water and immersed in solution sodium hydroxide (10 molar) for two minutes and then washed with tap water running for the next day (Abdul - Wahab, et, al, 1976) and then put them in a large bowl (30-50 cm) covered with a large filter paper moisturized with distilled water and then covered with another layer of filter paper and incubated at 30-35 C° for a period of 4 weeks was then obtained during the seedling roots along at a rate of 10-15 cm approx.
- Preparation acetone powder: separate roots from seeds and then placed directly in acetone for crud extract, mixed roots with acetone by ratio 1:10 (w/v) then mixed for 5 minutes by blender, and repeated the process twice and equally acetone volume under cooling conditions then filtered mixture using a Buchner funnel and filter paper installed on the vacuum pump . Wash the precipitate by adding sufficient amount of cooled acetone. Then drying under vacuum for several hours and saved for the purpose of extraction peroxidase .
- Extraction peroxidase enzyme: peroxidase extracted from of acetone powder of the roots (crude extract) from the last step by mixing with sodium acetate buffer solution by ratio 1:20 (w / v) .
- Determination peroxidase activity: enzyme activity determined according to method (Whitaker & Bernhard, 1972) by reaction of substrate consisting of O-dinazidine and hydrogen peroxide with a tris solution, and then measure the beginning of the reaction by adding 0.1 ml of enzyme extract, followed the change in the absorption at a wavelength 436 nm every 30 seconds for a period of 3 minutes for 7 readings .
- Purification of peroxidase enzyme: crud extract of soluble peroxidase obtained from acetone powder was purified by several steps included precipitation by ethyl alcohol with ratio ranged between (40-75 %) and then done ion exchange chromatography for enzyme solution by using column of ion exchange (D- Ethyl Amino Ethyl – Cellulose) dimensions (1.6 × 20 cm), which preceded equilibrated with solution buffer of potassium phosphate (0.005 molar) pH 8, then recovered enzyme solution with balance solution for unlinked parts with exchanger and gradient saline solution of sodium chloride ranged from (0 – 1 molar).

- Using peroxidase enzyme in determination of glucose:

A . Prepared solutions of glucose solution ranged between (1 -2 mg / ml) of standard glucose.

B . Diluted solution of O-dinazidine by 100 times buffer solution (pH 6) and ionic forces (0.15 molar) according to the method used by (Eills & Morrison, 1982) and consisting of three types of buffer solutions: Sodium acetate, Sodium phosphate and Tris, with continuous stirring (Tzouwara-Karayanni &Crouch,1990).

C . Added 0.5 ml of glucose solution prepared previously, blood serum and grape juice after diluted to 2.4 ml with solution of O-dinazidine in cell reaction and then added 0.1 ml of purified peroxidase (enzymatic activity 6 units / ml) then added 0.2 ml of a solution of purified glucose oxidase enzyme (enzymatic activity 200 units / ml) and then read on the absorbance at wavelength 436 nm (Whitaker & Bernhard,1972).

RESULTS AND DISCUSSION

Followed different methods and means to extraction and purification peroxidase enzyme from its sources, results listed in Table 1 shown that extraction of soluble peroxidase by buffer sodium acetate solution (1 molar) at pH 6 from acetone powder of roots was the best method compared with others results of peroxidase activity in the crude extract was 296400 units / g of acetone powder of roots, while peroxidase activity was 156750 units / g of acetone powder of leaves. The superiority of this method used for extraction due to the high efficiency of the extraction due to the nature of the tissues of the palm and its fibrous composition which was characterized with high resistance to cracking and crushing (Al-Bakir and Whitaker, 1978). These results agreed with results obtained about low activity for peroxidase from date leaves and other plant tissues which extracted by buffer solutions with different ionic forces (Baaziz and Saaidi, 1988). Moreover, the high specific activity for enzymatic extracts of acetone powder of roots and leaves by increase the ionic strength of the solution extraction return to the nature of the links enzyme with cell components (Yoon, et.al, 1993), which requires increasing the ionic strength of the extraction solution to realized those linkages emerging between the enzyme and pictic substances or others cellular parts (Silva, et. al, 1990).

Soluble peroxidase enzyme was purified by steps included concentrated by precipitation with ethyl alcohol (40-75 %), results listed in table 2 showed that the number of purification times was 1.89 and enzymatic recovery increase to 44.4% . Next step was dialysis of enzymatic solution versus distilled water to discard of the remnants of alcohol and some insoluble compounds reaching the number of the purification times to 2.9 with enzymatic recovery 34.4 %, followed by the step of ion exchange chromatography by column of di- ethyl amino ethyl cellulose (20 × 1.6 cm) enzymatic solution was recovered by balance buffer solution of potassium phosphate (0.05 molar) at pH 7.8, where increased the number of purification times to 4.92, with enzymatic recovery 29.6%, then concentrated and filtered through column Sephadex - G25, reached to the number of purification times 7.5 and enzymatic recovery 27.6 % .Linked enzyme recovered by linear gradient of saline solution of sodium chloride concentrations ranged from (0 - 1 molar).

Figure 1 shown appearance one peak of the protein and enzymatic activity in parts unlinked with Di diethyl amino ethyl - cellulose (Wash) and this indicate exit enzymatic solution of soluble peroxidase and similar proteins to the charge of exchanger, and the appearance of several peaks of protein and one peak and some enzymatic isomers which oboists charged to exchanger, as the peak of the protein be identical with enzymatic activity peak of the parts unlinked with exchanger be considerable as indicator for enzyme purity (Whitaker, 1972).

Peroxidase was used in estimating glucose concentration as an easy, fast method sharing with the glucose oxidase enzyme (GOD), and due to the high affinity shown by purified soluble enzyme towards O- Dinazidine that encouraged their for using in the estimation sugar enzymatically in the blood and food (Dalali, 1983).

CONCLUSIONS

1. Possibility of benefiting of the seeds dates (by-products) after manufacturing dates syrup and honey dates, for preparation peroxidase enzyme in large quantities .
2. Adoption method for preparation acetone powder of palm seeds roots as optimal method to obtained for extracts with high content of enzymes .
3. Using enzymatic methods as one of the methods used in determination of sugars which found to be the best of coloring methods specialized with sugar, such as enzyme glucose oxidase and peroxidase.
4. Results showed that purified peroxidase from plant sources besides the extract from the roots of palm seeds have high affinity towards O-dnazidine (as a substrate) and hydrogen peroxide where Quicken enzymatic reaction .
5. The possibility of exploiting the seeds roots of date (Zahdi date) as a source for the production of peroxidase enzyme in large quantities for analytical purposes and diagnostic immunohistochemistry (ELISA)) under controlled laboratory conditions as an alternative for local horseradish peroxidase HRP is available from one of the byproducts of the palm .

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Tables

Table 1: Comparison between specific activity (unit/gm) for soluble peroxidase enzyme and for extracted of roots and leaves for Zahdi date palm.

Code Method	Specific activity for soluble peroxidase enzyme from roots (unit/gm)	Specific activity for soluble peroxidase enzyme from leaves (unit/gm)
A	42180	23810
B	88920	34723
C-1	67260	38691

Code Method	Specific activity for soluble peroxidase enzyme from roots (unit/gm)	Specific activity for soluble peroxidase enzyme from leaves (unit/gm)
C-2	132240	77382
C-3	164160	82343
C-4	245100	156759
C-5	296400	113693
	188100	65477

- A: Direct extraction by buffer acetate solution (0.5 M).
- B : Direct extraction by buffer acetate solution (1.0 M).
- C-1: Extraction by sodium acetate buffer solution (0.1 M) of acetone powder of roots.
- C-2: Extraction by sodium acetate buffer solution (0.1 M),sodium chloride solution (0.5M) .
- C-3: Extraction by sodium acetate buffer solution (0.6 M) of acetone powder of roots.
- C-4: Extraction by sodium acetate buffer solution (0.8 M) of acetone powder of roots.
- C-5: Extraction by sodium acetate buffer solution (1.0 M) of acetone powder of roots.
- C-6: Extraction by sodium chloride solution (1.0 M) of acetone powder of roots.

Table 2: Purification steps of soluble peroxidase enzyme extracted from seeds root of Zahdi date.

Enzymatic recovery	Purification times	Total activity (unit)	Specific activity (unit/ml)	Protein (mg/ml)	Enzymatic activity (unit/ml)	volume (ml)	Purification steps
100	1	561600	34361.4	0.454	15690	36	Crud extract
44.4	1.89	249600	65271.9	0.478	31200	8	Precipitation with alcohol (40-70%)
34.4	2.91	193200	100311.5	0.321	32200	6	Dialysis against dis.water
29.6	4.92	166500	168965	0.058	9800	17	Ion exchange
2.24	0.21	12600	7200	0.070	501	25	Recovery
27.6	7.52	156017.5	258375	0.08	352	15	Gel filtration Sephadex G25

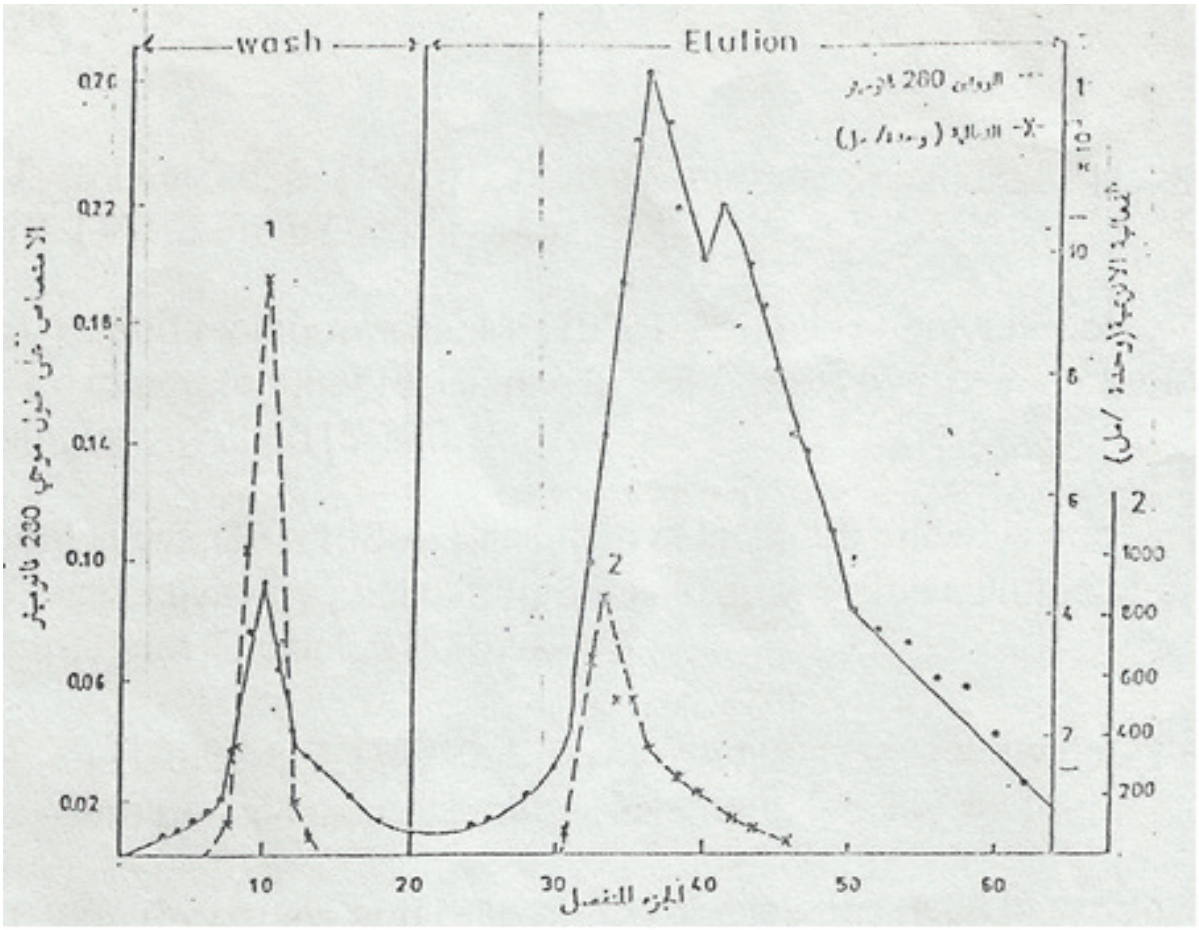

Figure 1: Ion exchange step

Detection and relevance of minor amino acids and amino components in Saudi date fruits

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Key words: *Phoenix dactylifera* L.; non-protein amino acids; D-amino acids; biogenic amines, plant amino acids; GC-MS; LC-MS; nutritional and health aspects

INTRODUCTION

Whereas an abundance of literature is available on free and protein-bound amino acids (AAs) in date fruits of *Phoenix dactylifera* L., reports on minor and special non-protein AAs or amino components are rather scarce.

We have analyzed extracts and acidic hydrolysates of 12 Saudi date fruits harvested at the 'Tamr' stage, representing full ripeness. With emphasis on the occurrence of such compounds we employed ion-exchange chromatography in the high-performance physiological mode, HPLC using pre-column derivatization with fluorescent AccQ reagent, gas-chromatography (GC) using isotopic standards, and chiral capillary GC of ^{2}HCL hydrolysates on the chiral stationary phase Chirasil-L-Val™

RESULTS

Besides common protein amino acids, the non-coded amino acids 5-hydroxy-pipecolic acid (5-OH-Pip), 4-hydroxyproline (Hyp), 1-aminocyclopropane-1-carboxylic acid (Acc), -aminobuyric acid (Gaba), -alanine, and the amino alcohol 2-aminoethanol (Eta) were detected. Evidence was also found for the presence of 5-hydroxylysine (5-OH-Lys). Enantiomeric resolution of L- and D-AAs on Chirasil-L-Val revealed the presence of trace amounts (1-3% D-AAs relative to L-AAs) of the optical antipodes of common L-AAs, namely D-aspartic acid, (D-Asp), D-Ala (D-Ala), and D-glutamic acid (D-Glu).

CONCLUSIONS

In plants, Acc is precursor of the plant hormone ethylene and quantities might serve as indicators for date ripeness. -Ala is used for the synthesis of panthotenic acid (vitamin B5). In humans, Gaba plays an important role as neurotransmitter. It is reported that food supplements fortified with Gaba have calming effects and promote sleep. Therefore, food rich in Gaba is recommended as natural tranquilizer. Eta modulates the rate of rat hepatocyte proliferation *in vitro* and *in vivo*. In the past focus was on possible negative effects of 'unnatural' D-AAs in foodstuffs. This point of view has changed entirely. The sodium salt of D-Asp is used as a commercial drug to improve semen quality and testosterone level of man. D-Ala is added to antipsychotics for the treatment of schizophrenia. Consequently, thorough study of date fruits regarding minor non-protein amino acids and amines using sophisticated state-of-the art analytical techniques will provide new insights in possible health benefits of date fruits not yet taken into account.

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