Proceedings of the Sixth International Date Palm Conference

Editors

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Ghaleb Alhadrami

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FOREWORD

I am pleased to introduce these proceedings for the Sixth International Date Palm Conference which 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 Khalifa International Award for Date Palm and Agricultural Innovation in collaboration with Ministry of Presidential Affairs, United Arab Emirates University, International Center for Biosaline Agriculture, Al Foah Company, Abu Dhabi Food Control Authority, Food and Agriculture Organization of the United Nations, Date Palm Friends Society, Date Palm Global Network, Arab Organization for Agricultural Development and International Center for Agricultural Research in the Dry Areas.

The conference was attended by government officials, ambassadors of the diplomatic corps accredited in UAE, representatives of international educational and research institutions, scientists, technicians and private date growers. More than 400 participants from about 40 countries attended the conference.

The conference objectives were to provide an opportunity for updating scientific information on different aspects of date palm production, propagation, protection, and marketing. Compare the recent experiences in the United Arab Emirates with those of other date growing countries, and foster international technical cooperation on different aspects of date palm production chain, were also the main aims of the conference.

The research resulting from this conference, and included in these proceedings, demonstrates the value of research and creative activities to social and economic development. It enhances our understanding of the place of the date palm in a modern world and helps foster regional and international collaboration. It will also enhance the economic value of the date palm and will promote its place in agricultural development. I am hopeful that these research articles will help make the field of Date Palm Research and Development an attractive one to new generations of scientists, technicians, and entrepreneurs.

I congratulate the authors and the contributors to these proceedings. I am hopeful that their excellent work will focus attention on the important role of universities and research centers in serving the needs of their communities.

Nahayan Mabarak Al Nahayan
Minister of Tolerance, President of Board of Trustees of
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PREFACE

The Proceedings of the Sixth International Date Palm Conference are published by Khalifa International Award for Date Palm and Agricultural Innovation. 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 these Proceedings of the Sixth International Date Palm Conference.

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

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Genetic Engineering and Biotechnology / Tissue Culture

Metabolomic approaches applied on the analysis of fruits and products of the date palm *Phoenix dactylifera* L.

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Abstract

Metabolomics is defined as the methodology for the entire profiling of low molecular compounds occurring in microorganisms, plants, animals and human beings. If applied to raw or processed foodstuffs the approach has been coined 'foodomics'. In previous work we had determined protein and non-protein amino acids in fruits of the date palm of different cultivars at varying ripening states. Here we focuse on date fruit-derived foodstuffs such as date syrup, date paste and date vinegar. In order to perform metabolic profiling of date fruits we used the 'chloroformate' approach, i.e. derivatization of protic compounds using ethylchloroformate/ethanol/pyridine and analysis of the resulting volatile derivatives using high-resolution GC-MS. Besides protein and non-protein amino acids the method enabled the derivatization of mono- and dicarboxylic acids, fruit acids, and fatty acid acids.

Besides IEC and GC-MS we applied hydrophilic interaction liquid chromatography combined with positive and negative ion electrospray ionization mass spectrometry to the aforementioned foods. Based on retention times and diagnostic fragment ions we could characterize metabolites such as betaine, choline, isomers of glucose and sucrose, deoxy-fructose diastereomers of proline and GABA, C16 and C18:2 fatty acids and citric acid as well as proteinogenic and non-proteinogenic amino acids such as GABA and *trans*-5-hydroxy-L-pipecolic acid (5-OH-Pip). Since 5-OH-Pip is very rare in edible fruits, this non-metabolizable amino acid is proposed as reliable authenticity and dietary biomarker for date fruits and products. Metabolomics based on high-resolution mass spectrometric methods are considered as the most advanced analytical methods for the comprehensive metabolic profiling of date palm fruits and foodstuffs made thereof.

Keywords: Foodomics*, trans*-5-hydroxypipecolic acid, food authentication, biomarker, chiral gas chromatography - mass spectrometry, liquid chromatography- mass spectrometry

Abbreviations

Common amino acids are abbreviated according to three-letter notation; Pip(OH) or 5-OH-Pip, *trans*-5-hydroxy-L-pipecolic acid; GABA, *gamma*-aminobutyric acid; C15, C16:0, C18:0, C18:1, C18:2n6 refer to common saturated and unsaturated fatty acids; GC-MS, gas chromatography - mass spectrometry; GC-SIM(TIC)-MS, gas chromatography selection ion monitoring (total ion current) mass spectrometry; IEC, automated amino acid analysis based on ion-exchange chromatography and ninhydrin derivatization; TMS, trimethylsilyl; EtCF, ethyl chloroformate; EtOH, ethanol; HFBCF, heptafluorobutyryl chloroformate; HFBOH, heptafluorobutanol; PESI (NESI) HILIC LC-HRMS, positive (negative) electrospray ionization - hydrophilic liquid chromatography high-resolution mass spectrometry.

INTRODUCTION

In previous work we had reported on the determination and stereochemistry of proteinogenic and non-proteinogenic amino acids in acidic hydrolysates of cultivars of Saudi Arabian date fruits (Ali et al., 2014. Besides common protein amino acids, a fair number of non-proteinogenic amino acids were detected and quantified such as *β*-alanine, *β*-aminoisobutyric acid, 5-hydroxylysin, ornithine, gamma-aminobutyric acid and pipecolic acid. For quantification mainly automated ion-exchange chromatography (IEC) employing the physiological mode and post-column derivatization with ninhydrin were used.

An unexpected result was the detection of large amounts of *trans*-5-hydroxypipecolic acid (abbreviated Pip(OH) in the following) in all date fruit hydrolysates, ranging from about 1.4 g to 4.0 g per kg dry matter. In contrast to Pip(OH) that was detected in all date samples and products analyzed, tentatively assigned 1-aminocyclopropane-1-carboxylic acid (Acc) in former work (Ali et al., 2014) could not be confirmed in any of the samples analyzed in this work. Thus, Acc is definitely not a significant component of date fruits. Pip(OH), notably occurring in the free state in date fruits, was most abundant among amino acids in hydrolysates. Since 5-OH-Pip is very rare in edible fruits, this non-metabolizable amino acid is proposed as reliable authenticity and dietary biomarker for date fruits and products.

In extension of our previous work and in order to get insight in the metabolic profiles of date fruit cultivars, we analyzed dates and date fruit-derived products employing an amino acid analyzer, gas-chromatography coupled with mass-spectrometry, and HILIC liquid chromatography coupled with mass spectrometry. The analytical approach aimed at the entire profiling of low molecular compounds occurring in organisms is named ‘metabolomics’. If applied to raw or processed foodstuffs the approach has been coined ‘foodomics’ (Cifuentes, 2012; Hu and Xu, 2013). Here we focus on fruit-derived foodstuffs such as date paste, date syrup and date vinegar. Besides high-resolution chiral and non-chiral GC-MS of derivatives, we applied hydrophilic interaction liquid chromatography combined with positive and negative ion electrospray ionization mass spectrometry to the aforementioned foods. Based on retention times and diagnostic fragment ions we could characterize metabolites such as betaine, choline, glucose and sucrose isomers, deoxy-fructose diastereomers of proline and GABA, C16 and C18:2 fatty acids and citric acid as well as proteinogenic and non-proteinogenic amino acids such as GABA and *trans*-5-hydroxypipecolic acid (5-OH-Pip). Since 5-OH-Pip, according to our present knowledge, is very rare in edible fruits, this non-metabolizable amino acid is proposed as reliable authenticity and dietary biomarker for date fruits and products. Metabolomics based on high-resolution mass spectrometric methods are considered as the most advanced analytical methods for the comprehensive metabolic profiling of date palm fruits and foodstuffs made thereof.

MATERIALS AND METHODS

Date fruits (cultivars ‘Ajwa al Madina’ and ‘Nabtat Ali’), date pastes, and date syrups were purchased from retail outlets in the vicinity of Riyadh, KSA and characterized in the following by corresponding numbers, i.e. date syrup no. 1 (brand ‘Basra’) and date paste no. 6 (see Figure captions). Date vinegar no. 1 (brand “Durra”) was a product of the Kingdom of Jordan.

Treatment of foodstuffs for analysis

Freeze dried date fruits (‘Ajwa al Madina’, no. 1 and ‘Nabtat Ali’ no. 2) were totally hydrolyzed in 6 M HCl and subjected to IEC as described (Ali et al., 2014). Syrups, pastes and vinegars were diluted with pH 2.2 buffer and analyzed automatically by IEC as described previously in detail (Ali et al., 2014).

For gas-chromatographic analysis aliquots were diluted with ethanol and derivatized at room temperature using ethyl chloroformate with addition of pyridine. For chiral amino acid analysis of vinegar no. 1 derivatization with HFBCF/HFBOH and a chiral capillary column (Chirasil-Val) were used. Derivatives were extracted into isooctane and injected into the capillary of the GC-instrument. For details see Šimek et al. (2012).

Instruments

For automated amino acid analysis, a 'Biotronik' analyzer run in the physiological mode was used (Ali et al. 2014). For GC-MS analysis of derivatives resulting from chloroformate derivatization a GC 1090 instrument with mass specific detector and a capillary column (Šimek et al., 2012) were used. Assignment of peaks was based on retention times and selected diagnostic fragment ions in comparison to a standard mixture.

For UHPLC-MS a dedicated HILIC column coupled to an Orbitrap Q HRMS instrument (Thermo Finnigan) were used and analyses performed as described by Koštál et al. (2011, Supplement). UHPLC was realized using a gradient of mixtures of acetonitrile/water with addition of formic acid. Mass spectra were measured in the positive and negative ionization mode. Assignment of compounds was based on retention times and molecular ions in comparison to standard mixtures.

RESULTS

Sections of chromatograms resulting from IEC of date fruit no. 1, syrup no. 1, date paste no. 6 and date vinegar no. 1 are shown in Figs. 1a-e. Pip(OH) was assigned in all foodstuffs and presence confirmed by GC-MS and LC-MS methods (see below). Presence of Pip(OH) in IEC chromatograms of total hydrolysates of date fruits no. 1 ('Ajwa al Madina') and no. 2 ('Nabtat Ali') was confirmed by GC-MS of trimethylsilyl derivatives and diagnostic fragment ions at *m/z* 244 and *m/z* 154 (Fig. 1f).

Sections of chromatograms resulting from the derivatization of components occurring in date syrup no. 1 and date paste no. 6 using the EtCF/EtOH approach are shown in Fig. 2. Besides most abundant Pip(OH) common amino acids could be assigned as well as fruit acids and saturated and unsaturated fatty acids. Detection of an abundance of further compounds is not discussed in this work. For the detection and separation of L- and D-amino acids in date vinegars, another chloroformate approach was used, namely derivatization with HFBCF/HFBOH. GC-MS on the chiral capillary column Chirasil-L-Val revealed the presence of relatively large amounts of certain D-amino acids (Fig. 3). Presence of D-amino acids in fermented foodstuffs is clear proof of bacterial fermentation. Together with the detection of Pip(OH) in this vinegar (see Fig. 1), presence of large quantities of D-amino acids is clear proof of microbial fermentation of date fruits by acetic acid bacteria, mainly species of *Acetobacter* (Erbe and Brückner, 1998).

Analysis of date syrup no. 1 and date paste no. 6 using LC-ESI-MS in the positive and negative ion mode (in contrast to GC-MS requiring no derivatization), proved again the presence of abundant Pip(OH). Simultaneously additional components such as fruit acids and fatty acids could be determined. Notably, AMADORI compounds of Pro and GABA could be detected (Figs. 4a and 4b).

CONCLUSIONS

Advanced GC- and LC-MS methods will boost FoodOMICS, and MetabolOMICS will provide new insights in low molecular weight metabolites of date fruits beyond established components. Since non-proteinogenic trans-5-hydroxypipecolic acid is an abundant and characteristic amino acid in all date fruits this amino acid is proposed as authenticity and biomarker for dates fruit cultivars of *Phoenix dactylifera* L. and food products made thereof.

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REFERENCES

Ali, H.S.M., Alhaj, O.A., Al-Khalifa, A.S., Brückner, H. (2014). Determination and stereochemistry of proteinogenic and non-proteinogenic amino acids in Saudi Arabian date fruits. Amino Acids 46:2241-2257.

Ali, H.S.M., Pätzold, R., Brückner, H. (2010). Gas chromatographic determination of amino acid enantiomers in bottled and aged wines. Amino Acids 38:951-958.

Cifuentes, A. (2012). Review Article. Food Analysis: Present, Future, and Foodomics. ISRN Analytical Chemistry, 2012:1-16.

Erbe, T., Brückner, H. (1998). Chiral amino acid analysis of vinegars using gas chromatography – selected ion monitoring mass spectrometry. Z. Lebensm. Unters. Forsch. A, 207:400-409.

Hu, C., Xu G. (2013). Mass-spectrometry-based metabolomics analysis for foodomics. Trends in Analytical Chemistry 52:36-46.

Koštál, V., Zahradníčková, Šimek, P. (2011). Hyperprolinemic larvae of the drosophilid fly, *Chymomyza costata*, survive cryopreservation in liquid nitrogen. PNAS 108: 13041-13046.

Šimek, P., Hušek, P., Zahradníčková, H. (2012). Heptafluorobutyl chloroformate-based sample preparation protocol for chiral and nonchiral amino acid analysis by gas chromatography. In: Alterman and Hunziker (eds.), Amino acid Analysis: Methods and Protocols, Methods in Molecular Biology, pp. 137-152, chapter 13. Humana Press by Springer Science + Business Media, LLC 2012.

Figures

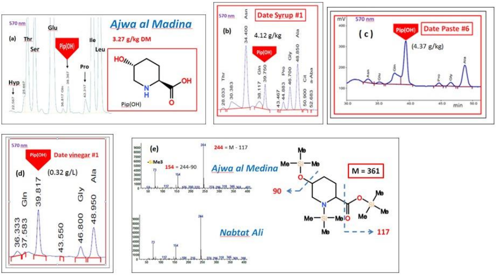

Fig. 1. Sections of IEC chromatograms of hydrolysed date fruit (a) 'Ajwa al Madina', (b) date syrup no. 1, (c) date paste no. 6, (d) date vinegar no. 1, and (e) GC-MS of TMS-derivatives confirming presence of *trans*-5-hydroxypipecolic acid in date fruit (a) and cultivar' Nabtat Ali'.

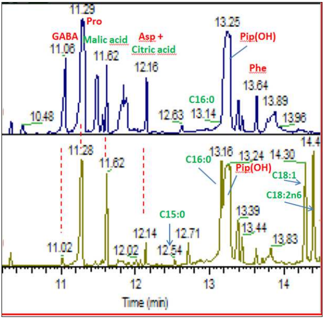

Fig. 2. Sections of chromatograms (GC-TIC-MS) resulting from EtCF/EtOH derivatives of date syrup no. 1 (top) and date paste no. 6 (bottom). For abbreviations see text.

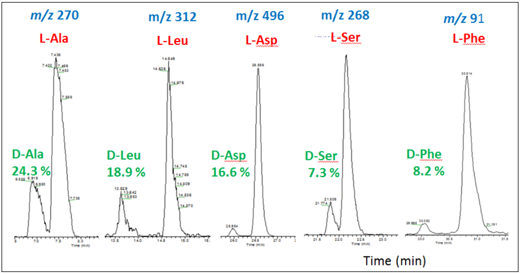

Fig. 3. Sections of chromatograms (GC-SIM-MS) and relative percentages (%) resulting from HFBCF/HFBOH derivatives of chiral amino acids detected in date vinegar no. 1, resolved on Chirasil-L-Val capillary column. Assignment of enantiomers is based on retention times and diagnostic fragment ions *m/z*.

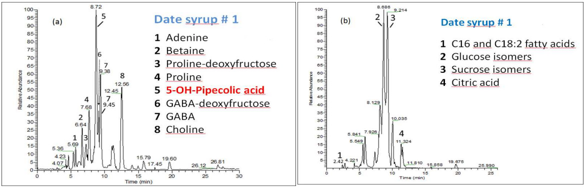

Fig. 4. (a) Positive-ion PESI HILIC LC-HRMS of $[M+H]^+$ ions of date syrup no. 1; (b) negative-ion NESI HILIC LC-HRMS of $[M]^-$ ions of the same date syrup no. 1. Assignment of ions is based on retention times of compounds and corresponding positive $[M + H]^+$ (left) and negative $[M]^-$ (right) molecular ions.

Microsatellites usage for standardizing cultivar identification in date palm

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Abstract

Selected microsatellite markers were evaluated for their potential in the fingerprinting of date palm cultivars in an attempt to standardizing identification procedures. Clonally propagated crops such as date palm, *Phoenix dactylifera* L., require that specific cultivars be correctly identified when initiating mass propagation. Periodic checks on the validity of the identity and their clonal trueness-to-type as they move through the micropropagation procedures of multiplication, acclimation and shipping in commercial propagation facilities is important for successful date palm propagation. Recent work has been successful in the sequencing of two cultivars within the date palm genome and various procedures that facilitate "fingerprinting" of specific genotypes. Simple sequence repeat (SSR) panels, accessible microsatellites in multiplex PCR, was used to successfully identify date palm cultivars. Four primer pairs, amplifying loci represented as primer sequences with appropriate multiplex 5' modification, were used to fingerprint 56 date accessions representative of important globally commercial available cultivars. These accessions were subjected to evaluation and demonstrated sufficient polymorphism to distinguish among 54 of the cultivars. A total of 38 (33 females and 5 males) could be confirmed in their identity by the trilateral confirmation system through experts and morphological characteristics, the remaining 18 represented cultivars that were new and potentially different. Results suggest that much greater intra-varietal diversity exists within the collected populations as allelic composition was well conserved among the varietal sub-groups. Further microsatellite markers or SNPs will be required to fingerprint the remaining 18 genotypes and others from a genetically more variable array. Additional motifs for the detection of multiple polymorphic fragments will be needed to cover larger panels and populations of diverse backgrounds among date cultivars. These results show that a relatively few microsatellites can be used to confirm large numbers of specific cultivars and thus provides a valuable and affordable tool for verification of genotypes in the mass propagation of date palm for commercial purposes.

Keywords: Cultivar, Identification, Date palm, Simple sequence repeats, Palmàprinting, Laboratory quality control, Fingerprinting, *P. dactylifera* L.

Fingerprinting of some Omani date palm cultivars using SSR markers

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Abstract

The numbers of known date palm cultivars that are distributed all over the world are approximately 5,000, out of which about 250 are found only in Oman. It is a dioecious, perennial, monocot plant, and its heterozygous form makes its progeny strongly heterogeneous. Most of these cultivars were described using morphological markers such as fruit and vegetative traits, but these are greatly affected by the environment and are also complex. In general, the identification and evaluation of genetic diversity between the cultivars on the basis of morphological markers is difficult. Recently DNA markers have been used to provide the information on the relatedness of date palm cultivars that are difficult to distinguish morphologically. Microsatellites (SSR) have been used widely in date palm genetic diversity among GCC countries. In this study, the genetic diversity of twelve of Omani date palm cultivars was studied by using 10 microsatellite markers which is same markers used in GCC countries date palm cultivars. Mature leaves samples of all cultivars, five replicates per cultivar, were collected from five governorates of Oman from north and south Al-Batinah (Sohar and Barka), Al-Dhahirah (Ibri), Al-Dakhilia (Wadi Quriate, Nizwa), Al-Sharqiya (Samad Al-Shan, Al-Kamel & Al-Alwafi) and Al-Burymi (Mahadah, Al-Buraymi). A total of 113 alleles were scored with average of 11.3 alleles per locus. It was ranged from 5 alleles/locus for SSR (PDCAT 17) to 17 alleles/locus SSR (mPdCIR 10). The polymorphic information content (PIC) average 0.668. There was genetic diversity within and among the selected cultivars and were assessed by using microsatellite markers.

INTRODUCTION

Microsatellite or simple sequence repeat (SSR) molecular markers have been proven to be very powerful in plant diversity analysis because they are locus specific, co-dominant, highly polymorphic and highly reproducible. Genetic variation in the date palm germplasm has been traditionally characterized using morphological descriptors. However, such morphological markers are often unreliable and ambiguous because of the influence of environmental factors and confounding effects of developmental stage of the plant) Barrow, 1998). The aim of this investigation was to study the genetic variation (polymorphisms) among different Omani date palm cultivars using SSR markers. Date palm (*Phoenix dactylifera* L.) is a dioecious perennial monocotyledon plant with long generation times (a period of 4 to 5 years is necessary to reach the first flowering) that belongs to *Arecaceae* family (Elhoumaizi, 2002). It is important to study the genetic diversity of the Omani date palm cultivars, because it helps to find the identity of DNA that will helps in documenting the Omani cultivars in order to preserve them. Also, to determine the strains within species, make sure and check conventional seedlings before being placed in the tissue propagation, definition promising new cultivars for farmers, to make sure the genetic stability of the output of agriculture and textile version genetic compatibility with the original certification and to ensure product quality.

The most common characteristics that are used to identify different cultivars of date palm are the morphology of leaves, spines, and fruit, which are mainly based on the characterization of introduced date palm cultivars in California (Nixon, 1950). Over the years, many date palm cultivars have been transplanted to areas other than the area of their origin, and they may have been given different names. As a result, a variety may have different names in different areas, or two genetically different varieties may have the same name. This may reduce the genetic diversity of the cultivars, making them vulnerable to biotic and abiotic stresses. In general, the identification and evaluation of genetic diversity between cultivars on the basis of morphological markers is difficult. The identification of trees is usually not possible until the onset of fruiting, which takes 3 to 5 years. Further, characterizing varieties requires a large set of phenotypic data that are difficult to access statistically and are variable because of environmental effects (Sedra *et. al.*, 1993, 1996, 1998). Biochemical markers (isozymes and proteins) are effective in varietal identification (Bennaceur *et. al.*, 1991; Fakir et al., 1992; Bendiab *et. al.*, 1993). However, they give limited information and are an indirect approach for detecting genomic variation (Elmeer, 2015).

In this study, we aimed to investigate the genetic diversity of date palms in Oman to improve production and reveal the genetic relationships among 10 date palm cultivars using 12 nuclear microsatellite markers. These cultivars have names that were given by farmers after continuous selection.

MATERIAL AND METHODS

Collection of material

Date palm material was collected 5 replicate of 12 cultivars from the different region on Oman (North and South Al-Batinah, Al-Dhahirah, Al-Dakhliah, Al-Sharqiah, Al-Buraimi governorates) as shown in Table 1 and Fig. 1. These cultivars represent the diversity of date palm genotypes in the Omani date palm plantation. Young leaves from mature, randomly sampled trees, were collected and stored at -80°C, until DNA extraction.

Molecular analysis

DNA was extracted by using (Maxi: kit Qiagen Cat # 68163 DNeasy plant). It was measured DNA concentration using a 1% of Agarose gel and then detected using UV light and was used the device (Nanodrope) to ensure the purity of DNA by absorption measurement at a wavelength between 260 and 280 nm to enter it in The next stage figure2. The microsatellite amplification reaction was performed by using Applied Biosystems (2720 thermo cycler, Singapore) with 10 primers combinations. Table 2 shows the microsatellite combination and its allelic ranges (Peakall 2012). The PCR program had initial denaturation at 95 ^{0}C for 5 minutes, then 35 cycles of 95 ^{0}C for 30 minutes, 52/55 ^{0}C for 1 min, and 72 ^{0}C for 1 minute and final elongation step at 72 ^{0}C for 7 minutes. Amplification products were separated using 2% agarose gel electrophoresis shown figure3. The microsatellite alleles were detected using Beckman coulter CEQTM 8000 automated DNA sequencer machine. Control sample in replication was used in this experiment along with the samples to be analyzed, to ensure the repeatability and accuracy of results.

RESULTS AND DISCUSSION

The targeted fragments and allele scoring were performed by fragment analysis. For each marker, the average number of alleles per locus, the expected heterozygosity (He) and the observed heterozygosity (Ho) were calculated by Gene Alex 6.3 software. The genetic similarity and the analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA) were also calculated using Gene Alex 6.3 software. DARwin 6.0 software was used to make dendrogram which showed the distribution of different individuals.

A total of 113 alleles were scored with average of 11.3 alleles per locus. It was ranged from 5 alleles/locus for SSR (mPdCIR 57) to 20 for locuse SSR (mPdCIR 10). The polymorphic information content (PIC) average .6650 and it was range between 0.4818 (PDCAT21) and 0.9125 (mPdCIR 10) (Table 3). The average of expected heterozygosity (He) ranged between 0.227 (mPdCIR 85) and 0.718 (mPdCIR 10) and the average of observed heterozygosity (Ho) ranged between 0.150 (mPdCIR 85) and 0.700 (mPdCIR 16) (**Table 3**). Most of markers, the observed heterozygosity value was higher than the expected one. The percentage of polymorphic loci per cultivar varied between 60% and 100% with an average of 85% (**Table 4**). Molecular variance analysis showed that 70 % of the variation was due to differences within populations, while 30 % was due to differences between populations in Omani cultivars (Fig. 4).

The Dendrogram shown in figure 5, illustrates the divergence between the studied Omani date palm cultivars and suggests their tree brunching, Dendrogram divided into four main groups, Group A has the following cultivars (Lolo, Menaz, Kash Qantrah, Shahel, Hilali Makran). While Group B has the following cultivars (Selani, Hasas, Manhi) and group C has the following cultivars (Nasho Al-khashba, Hilali Asfer, Merzaban, Hilali A-Hasa) and group D has combination with different following cultivars: Hilali Alhasa, Shahel, Merzaban (Fig. 6). The principal coordinates analysis (PCoA) of the 12 Omani cultivars showed that the majority of cultivars were grouped in cluster and also dispersed among different sub-clusters. DNA SSR markers are powerful tool to provide information on the relatedness of varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. In this study, SSR markers have been used to assess the molecular characterization and the phylogenic relationships of Omani date palm cultivars. Present results provide evidence of a genetic diversity

CONCLUSION

This study showed the distribution of Omani date palm cultivars from different region in Oman and analysis based on SSR markers. In future s this technique will help our study and provide a useful tool for research on genetic diversity, gene mapping, and marker-assisted selection in date palm. Therefore, while allowing studies on genetic variation, SSR markers also provides information on gene function related to possible phenotypic differences between the date palm cultivars.

References

Barrow S. 1998. A monograph of Phoenix L. (*Palmae: Coryphoideae*). Kew Bull 53: 513–575.

Bendiab K., M. Baaziz, Z. Brakez and M.H. Sedra. 1993. Correlation of isoenzyme polymorphism and Bayouddisease resistance in date palm cultivars and progeny. *Euphytica* 65:23-32

Bennaceur M., C. Lanaud, M.H. Chevalier and N. Bounagua. 1991. Genetic diversity of date palm (Phoenix dactylifera L.) from Algeria revealed by enzyme markers. *Plant breed* 107:56-69.

Elhoumaizi M.A., M. Saaidi, A. Oihabi and C. Cilas. 2002. Phenotypic diversity of date-palm cultivars (*Phoenix dactylifera* L.) from Morocco. *Genet. Resour. Crop. Evol*. 2002; 49: 483–490. doi: 10.1023/A:1020968513494.

Elmeer K. and I. Mattat. 2015. Genetic diversity of Qatari date palm using SSR markers. Genetics and Molecular Research, 14(1), pp.1624-1635.

Fakir S. and P. Munier. 1981. Origine de la culture sur palmier dattier et sa propagation en Afrique. Fruits 36 (437-450).

Nixon R.W. (1950). Imported Cultivars of Dates in the United States. United States Department of Agriculture Circular No. 834, Washington, D.C.

Peakall R. and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28: 2537-2539.

Sedra M.H., H. Filali and D. Frira. 1993. Observations sur quelques caracteristiques phenotypiques et agronomiques du fruit des varieties et clones du palmier dattier selectionnes. Al Awamia 82: 105-120.

Sedra M.H., H. Fila, A. Benzine and M. Allaoui. 1996. La palmeraie dattière marocaine : évaluation du patrimoine phenicicole. Fruits 1: 247-259.

Sedra M.H., Lashermes P., Trouslot P. and M.C. Combes. 1998. Identification and genetic diversity analysis of date palm (Phoenix dactylifera L.) varieties from Morocco using RAPD markers. Euphytica 103: 75-82.

Tables

Table 1. Samples used in fingerprint study

No	Sample Name	Code	Location	No. replicate
1	Selani	SL	North and South Al-Batinah, Al-Dhahirah, Al-Dakhliah, Al-Sharqiah, Al-Buraimi	5 each
2	Hasas	HSS		
3	Merzaban	MR		
4	Shahel	SHL		
5	Menaz	MNZ		
6	Manhi	MNH		
7	Lolo	LO		
8	Hilali Makran	HM		
9	Hilali Al-Hasa	HH		
10	Hilali Asfer	HA		
11	Nasho Al-Khashba	NK		
12	Kash Qantrah	KK		

Table 2. List of microsatellite primers designed for date palm by Billotte el al. (2004) and Akkak et al. (2009), marker name, annealing temperature Tm (^{0}), motif repeat, observed allelic size range (bp) and status of amplification.

No	Locus Name	Sequences (5 '-3')	Annealing T_m (^{0}).	Motif repeat	Expected Allelic range(bp)	Observed Allelic range(bp)
1	PDCAT10	F: CACTGCTCCTGTTGCCCTGT R: TGTAGAAGGGCAGAGGACGG	55°C	$(TC)_{16}$	107-127	114-128
2	PDCAT14	F: TGCTGCAAATCTAGGTCACGA R: GTTTACCCCTCGGCCAAATGTAA	55°C	$(TC)_{19}(TC)_{16}$	101-155	135-168
3	PDCAT17	F: CAGCGGAGGGTGGGCCTC R: GTTTCTCCATCTCCCTTTTTCTGCTACTC	55°C	$(GA)_{21}$	116-145	143-165
4	PDCAT20	F: TTTCAGACACATCAAGTAACGATGA R: GTTTACGTCCACCCCAAGTTACGA	55°C	$(GA)_{29}$	294-353	343-361
5	PDCAT21	F: GTGTTTGAAGATTGATTTTGTGTTATGAG R: GTTTCGAACTATGCACAATAGTATATTG	55°C	$(GA)_5T(GA)_2TA(GA)_2GC(GA)_5(GT)_7$	144-150	143-163
6	mPdCIR10	F: ACC CCG GAC GTG AGG TG R: CGT CGA TCT CCT CCT TTG TCT C	52°C	$(GA)_{22}$	118-161	130-152
7	mPdCIR15	F: AGC TGG CTC CTC CCT TCT TA R: GCT CGG TTG GAC TTG TTC T	52°C	$(GA)_{15}$	120-156	140-157
8	mPdCIR16	F: AGC GGG AAA TGA AAA GGT AT R: ATG AAA ACG TGC CAA ATG TC	52°C	$(GA)_{14}$	130-138	143-157
9	mPdCIR85	F: GAG AGA GGG TGG TGT TAT T R: TTC ATC CAG AAC CAC AGT A	52°C	$(GA)_{29}$	152-183	174-200
10	mPdCIR93	F: CCA TTT ATC ATT CCC TCT CTT G R: CTT GGT AGC TGC GTT TCT TG	52°C	$(GA)_{16}$	153-184	160-181

Table 3. The PIC values and alleles/loci of 17 microsatellite primer combination and Heterozygoty of Omani cultivars calculated with GenAlex 6.3 software He: average of expected heterozygosity; Ho: average of observed heterozygosity.

Locus Name	Alleles/loci	PIC Value	He	Ho
PDCAT10	10	0.639	0.627	0.683
PDCAT14	10	0.705	0.262	0.167
PDCAT17	5	0.573	0.375	0.483
PDCAT20	9	0.555	0.427	0.483

PDCAT21	7	0.548	0.420	0.517
mPdCIR 10	17	0.916	0.718	0.450
mPdCIR 15	15	0.879	0.672	0.683
mPdCIR 16	13	0.779	0.610	0.700
mPdCIR 85	11	0.570	0.227	0.150
mPdCIR 93	16	0.798	0.682	0.650
Total	113	-		
Average	11.3	0.668		

Table 4. Percentage of polymorphic loci of the studied Omani cultivars

Population	Percentage (%)
Selani	80
Hasas	70
Merzaban	90
Shahel	100
Menaz	90
Manhi	100
Lolo	60
Hilali Makran	90
Hilali Al-Hasa	80
Hilali Asfer	90
Nasho Al-khashba	80
Kash Qantrah	90
mean	85

Figures

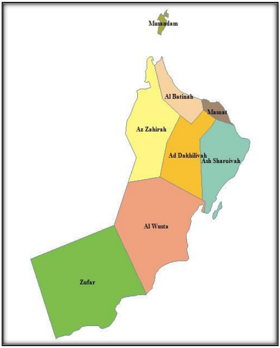

Fig. 1. Locations of Date palms collected

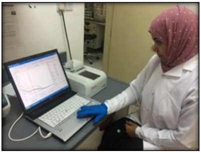

Fig. 2. Measuring the purity of the DNA using a device (Nanodrop).

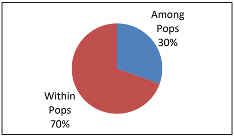

Fig. 3. Analysis of molecular variance between Oman cultivars.

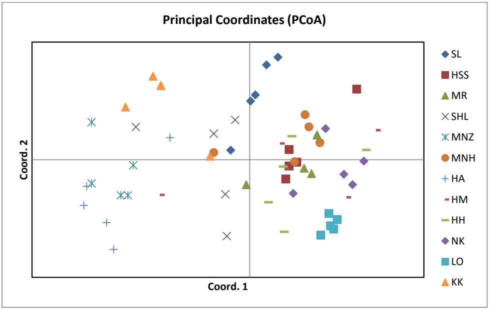

Fig. 4. Principal coordinates analysis of the studied Omani date palm cultivars

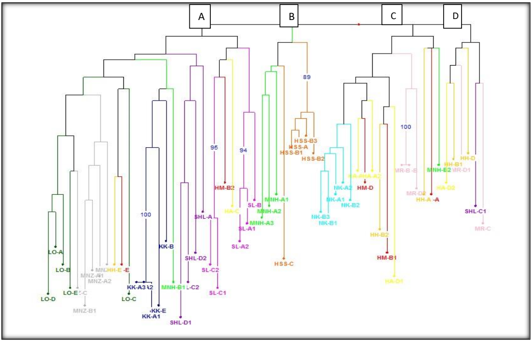

Fig. 5. Dendrogram of similarity coefficients based on UPGMA cluster analysis of 12 genotypes using 10 microsatellite (SSR) primer pairs**.**

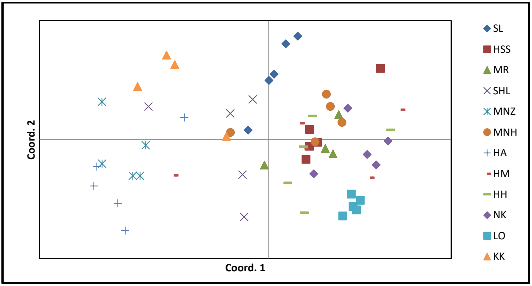

Fig. 6. Principal Coordinates Analysis (PCoA) of 12 Omani date palm cultivars

Genetic structure and diversity of commercially important date palm cultivars (*Phoenix dactylifera* L.) using phylogenetic relationships and simple sequence repeats (Microsatellites)

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Abstract

***Phoenix dactylifera* L. (the date palm) is the notable palm which produces a nutrient – rich edible fruit (the date), well known for its unique attributes of medicine and healthy energy. It is a species that has been cultivated since early civilizations in the fertile crescent and later in the Middle East. It is typically cloned with many cultivars. An investigation into their genomes and origins should provide valuable information for their maintenance and potential improvement of superior genotypes. Phylogenetic relationships amid commercial date cultivars are poorly understood, despite their importance. Here we report on the investigation of 20 selected, commercially important date palm cultivars consisting of 18 females and 2 males which are grown throughout the world. The knowledge of relationships among cultivars is needed, although the date palm genome has been mostly sequenced (90.2 %) with 41,660 gene models representing 82,354 scaffolds, remains unclear. Presently, the information on the characterization of these cultivars requires an assessment to better understand the relationships among the superior genotypes. The use of microsatellites, due to their accuracy and high polymorphic capability, have led to fine scaled phylogenies. The phylogenetic relationships were determined using neighbor joining un-rooted trees correlated with genetic clustering. Primer selections were achieved from evaluation of 14 nuclear Sample SSR loci isolated from *P. dactylifera* L. as reported by Billotte et al. Although one would anticipate a concentrated domestication because of clonal propagation, results revealed a high degree of polymorphism observed in the 20 cultivars with fewer common alleles than anticipated. Within the cultivars studied, a broad heterozygosity across bp amplification data has led to an understanding of limited inbreeding accounting for possibly adaptation to environmental changes. Population structure analysis suggests a large genetic boundary between Northwest African and the Middle East with 6 subpopulations that represent divergences and fragments of admixture in cultivars present in these regions. The possible selection of potential and good quality parents is achievable for improving cultivars by generating population and structure maps. This analysis documents patterns of relationship and provides genetic structure and cultivar specificity for this unique tree crop which should permit analysis of evolutionary relationships and information relative to population genetics.**

Keywords: Microsatellites, *Phoenix dactylifera* L., Simple sequence repeats, Phylogenetics, Genetic structure, Date palm.

Date Palm (*Phoenix dactyliferous* L.) Genetic Diversity and Conservation under Climate Change

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Abstract

Date palm (*Phoenix dactyliferous* L.) is an economically important tree species, grown in the arid and semiarid regions of the Middle East and North Africa. Recently, its cultivation has expanded to Australia, Southern Africa, South America, Mexico and the southwestern USA. Date fruits are highly nutritive, versatile tree byproducts, and diverse medicinal properties. There is an increase in demand of dates worldwide and requires to enhance date production by producing new improved cultivars, conservation, prevention, and utilization of spontaneous and induced date palm genetic diversity. Induced genetic diversity is caused by radiation and chemical mutagens. Climate change is a greater challenge on date production, e.g. water availability, soil quality, insect and pests. Date palm genetic diversity is conserved by cryopreservation (somatic embryos, embryogenic cell cultures), cold storage (seed and in vitro shoots) and in vivo (field gene banks), establish germplasm website for exchange and utilization. Plant regeneration from somatic embryos and embryogenic cell suspension is necessary for applying cryopreservation by using liquid nitrogen. In cold storage, shoot cultures are preserved at 4-5^{0}C, however subcultures are needed even though their number is reduced. In cryopreservation subculture is not needed and cultures are stored for longer period. Seed banks are commonly used in most of the seed crops. Field gene bank is alternate to in vitro conservation, and is being widely used, however has risk of insect and pest attack. This approach could be best applied under controlled conditions in plastic house. We will discuss the importance of different approaches of date palm gene pool conservation, climate change, and setting up of germplasm pool bank.

Keywords: Genetic diversity, cryopreservation, cold storage, field gene banks, seed bank, climate change

INTRODUCTION

In the past, global climate has continuously been changing, seems to continue to change in the coming future. Recently erratic and rapid climate change is being recorded worldwide that has affected food and agriculture especially developing countries have more devastated impact due to poor infrastructure, trained manpower, and economic conditions. Rapidly changing climate and ever-growing human population have caused loss of genetic resources, arable land and water shortage. Any small change in global temperature may develop new pests and disease that may devastate food and agriculture. The availability of sufficient water is another matter of concern to sustainable agriculture.

Genetic diversity is the key for the survival and evolution of species. Genetic variation within a species is important for its ability to adapt to a changing environment (Ahuja, 2017). Species having larger levels of genetic diversity have a better chance of adaptation, survival, and deployment over a wide range of environmental conditions. Appropriate levels of genetic variation should be maintained in the populations of a species for conservation planning. The conservation of genetic resources should be based on the genetic architecture and phenology, and how genetic and phenotypic variation is organized and distributed within and among populations of a species.

MATERIALS AND METHODS

Date Palm-A Tree of Life

The date palm (*Phoenix dactylifera* L.), a tree of life (Fig.1) - considered one of the most ancient plant cultivated in Mesopotamia some 4,000 years ago. It belongs to the monocot family Arecaceae; an arborescent, dioecious tall evergreen and highly heterozygous plant providing nutrition, as a staple food, food security, health benefits, shelter, raw material to the food industry, and fuel to the people. Even though date fruits are rich in nutrition, minerals, sugar and phytochemicals and its global market share is extremely low.

There are at least 15 minerals in dates, varies from 0.1 to 916 mg/100g, include boron, potassium, phosphorous, sodium and zinc. The seeds contain aluminum, cadmium, chloride, lead and Sulphur in various proportions. The total date palm production is 350,000 tons/year with an average yield 30-40 kg/tree. It is well distributed throughout the Middle East, North Africa, South Sahel, East and South Africa, and in certain parts of Europe and USA. This tree is an important economically fruit crop of the date palm growing countries. Date palm trees are high resilience and tolerance to environmental stresses- high temperature and radiation, low soil and atmospheric moisture, extended periods of drought, high salinity levels, and large diurnal and seasonal fluctuations. Date palm plantation creates an equable microclimate within oasis ecosystems and enables agriculture development as well as helpful in the conservation of the fragile environment structure and reduce desertification risks. It is an excellent source of edible sweet fruit in the arid and semi-arid regions worldwide. Date fruit ripening requires hot and dry climate with very low humidity and requires 200-250 mm rainfall. As the climatic conditions are continuously changing, rainfall during flowering and ripening stages may lead to considerable loss of fruit production and quality; lead to considerably economic loses impacting growers and provision of nutrition. Date palm is well known for highly nutritious fruits, versatile tree byproducts, and diverse medicinal properties.

Problems Facing Date Palm Cultivation

The date palm production is faced difficulties in sustainable production and proper utilization of available genetic diversity globally due to biotic and abiotic stresses (Zaid et al. 2002), industrialization, human development, and climatic variation. The loss of date palm plantations due human activities would need to be replaced with new plantations for sustainable production. Rainfall fluctuation has resulted in a gradual decline in ground water table and threatening survivability of communities in several date palm growing countries (Abul-Soad et al. 2017). The availability of water and impact on soil quality would deteriorate by 2100 (Shabani et al 2012). Pests and diseases invade date palm cultivation including Bayoud disease, caused by a fungus *Fusarium oxsysporum*f.sp. albedinis, has destroyed millions of date trees in North African countries Morocco and Algeria (Sedra 2011), however Tunisia has so far been safe from this disease. In addition, fungus *Fusarium solani* attacks date plan in Pakistan (Abul-Soad et al. 2017), and Al-Wijam disease caused by phytoplasma in Saudi Arabia (Alhdaib et al. 2007). The common symptom of these diseases is yellowing of feathered fronds followed by drying out to end by palm death. The major pest red palm weevil (*Rhynchophorus ferrugineus*) is rapidly becoming a big threat to date palm cultivation in India, Arabian Gulf countries, and Egypt in the North of Africa. This pest started spreading in early nineteenth century through shipment of adult seedling palm for landscaping purposes in the last decade. Also, drought, high salinity, over aged trees and genetic erosion are the major threats to combat with loss of date palm biodiversity globally.

Global Warming and Climate Change

Global warming can be defined as “the steady rise in the average temperature of the Earth’s atmosphere and oceans due to trapping of heat in the atmosphere by greenhouse gases. Climate change is a multidimensional and simultaneous variation in duration, frequency and intensity of parameters like temperature and precipitation, altering the seasons, melting of glacier (Fig.2) and life on the Earth. In this scenario, plant species with increased adaptive plasticity will be better equipped to tolerate changes in the frequency of extreme weather events.

The continuing increase in greenhouse gas emissions raises the temperature of the earth’s atmosphere. This results to melting of glaciers, unpredictable rainfall patterns, and extreme weather events. The accelerating pace of climate change, combined with global population and depletion of agricultural resources threatens food security globally. The overall impact of climate change as it affects agriculture was described by the Intergovernmental Panel on Climate Change (IPCC, 2007), and cited by the US EPA (2011) to be as follows:

- Increases in average temperature will result to: i) increased crop productivity in high latitude temperate regions due to the lengthening of the growing season; ii) reduced crop productivity in low latitude subtropical and tropical regions where summer heat is already limiting productivity; and iii) reduced productivity due to an increase in soil evaporation rates.
- Change in amount of rainfall and patterns will affect soil erosion rates and soil moisture, which are important for crop yields. Precipitation will increase in high latitudes, and decrease in most subtropical low latitude regions – some by as much as about 20%, leading to long drought spells.
- Rising atmospheric concentrations of CO2 will boost and enhance the growth of some crops but other aspects of climate change (e.g., higher temperatures and precipitation changes) may offset any beneficial boosting effect of higher CO2 levels.
- Pollution levels of tropospheric ozone (or bad ozone that can damage living tissue and break down certain materials) may increase due to the rise in CO2 emissions. This may lead to higher temperatures that will offset the increased growth of crops resulting from higher levels of CO2.
- Changes in the frequency and severity of heat waves, drought, floods and hurricanes, remain a key uncertain factor that may potentially affect agriculture.
- Climatic changes will affect agricultural systems and may lead to emergence of new pests and diseases.

Climate is changing and, as a consequence, some areas that are climatically suitable for date palm (*Phoenix dactylifera* L.) cultivation at the present time will become unsuitable in the future.

In contrast, some areas that are unsuitable under the current climate will become suitable in the future. Consequently, countries that are dependent on date fruit export will experience economic decline, while other countries’ economies could improve. Knowledge of the likely potential distribution of this economically important crop under current and future climate scenarios will be useful in planning better strategies to manage such issues. This study used CLIMEX to estimate potential date palm distribution under current and future climate models by using one emission scenario (A2) with two different global climate models (GCMs), CSIRO-Mk3.0 (CS) and MIROC-H (MR) (Shabani et al 2012). The results indicate that in North Africa, many areas with a suitable climate for this species are projected to become climatically unsuitable by 2100. In North and South America, locations such as south-eastern Bolivia and northern Venezuela will become climatically more suitable. By 2070, Saudi Arabia, Iraq and western Iran are projected to have a reduction in climate suitability. The results indicate that cold and dry stresses will play an important role in date palm distribution in the future. Also the climatic conditions (air temperature and humidity) and soil type play a significant role in the fruit properties of any cultivar during months of ripening. As the cv. 'Deglet Noor' develops black nose (blackening and shriveling of the tip) and fruit checks (small, linear scars near the apex) when grown in humid conditions. These results can inform strategic planning by government and agricultural organizations by identifying new areas in which to cultivate this economically important crop in the future and those areas that will need greater attention due to becoming marginal regions for continued date palm cultivation. Deglet Noor' is a native cultivar of Algeria and Tunisia where it performs as a dry cultivar while it is generally a semi-dry cultivar under USA environmental conditions (Krueger 2015). It is noticed that the soft cultivar of Egypt 'Samany' showed semi-dry fruit quality when cultivated in the date palm repository in USA. Moreover, under non appropriate environmental conditions, cv. 'Deglet Noor' yields, and quality were often unsatisfactory. Sometimes these differences happened even in small climatic differences in intra-zones. Date palms grown on hills or mountain range contains low moisture and subsequent having the longer shelf- life in comparison with same cultivar grown warmer and dry and plain areas. These results clearly indicated intra-cultivar variability due to environmental effects on fruit quality.

Climate Change and People Migration

Climate change adverse impact on people migration from rural to the urban areas in Vietnam. The Vietnamese Mekong Delta is one of Earth's most agriculturally productive regions and is of global importance for its exports of rice, shrimp, and fruit. The 18million inhabitants of this low-lying river delta are also some of the world's most vulnerable to climate change. The commune had lost its entire sugarcane crop after unexpectedly high levels of salt water seeped into the soil and killed the plants. Those without a safety net were living in poverty. Over the following weeks hundreds of smallholders, many of whom had farmed the delta for generations, were changing and their livelihoods would soon be untenable. In 2015-2016 disaster struck with the worst drought in a century. This caused salt water to intrude over 80km inland and destroyed at least 160,000ha of crops. In Kiên Giang (pop. 1.7m), one of the worst affected provinces, the local net migration rate jumped and in the year that followed around one resident in every 100 left. Climate change is the dominant factor in the decisions of 14.5% of migrants leaving the Mekong Delta. And its worth pointing out the largest factor in individual decisions to leave the Delta was found to be the desire to escape poverty. As climate change has a growing and complex relationship with poverty, 14.5% may even be an underestimate. All this demonstrates that climate change threatens to exacerbate the existing trends of economic migration. One large scale study of migration in deltas has found that climate factors such as extreme floods, cyclones, erosion and land degradation play a role in making natural resource-based livelihoods more tenuous, further encouraging inhabitants to migrate. Climate change could have impact on people migration in date palm growing countries,

Date Palm Genetic Diversity

Jaradat (2015) defined genetic diversity as the genetic variation between species, subspecies, cultivars, populations, or individual clones that can be measured morphologically, physiologically, biochemically, and at the molecular level. Date palm cultivars have evolved by thousands of years of seedling selection with desired characteristics and wide range of genetic diversity in fruit quality, and shape (Fig.3). Each cultivar is derived from a unique single seed, cloned and vegetative propagation (Adel-Soad et al, 2017). At the global level, over 5000 date palm cultivars exit in date palm growing countries, but sometimes might be synonyms of one cultivar found in different countries under a different name, but about 10% of them of a commercial importance (Johnson 2011). However, each country got its own top elite cultivars of commercial value. An Egyptian most famous cultivar 'Siwy' is widely cultivated in Siwa oasis. A Libyan date palm cultivar, Fruit of 'Saidi' is characterized with a brown ring made the fruit having two colors. This cultivar is also maintained in Date Palm Repository, USA.

Dates are categorized into soft, semidry, and dry cultivars as per their moisture content, texture, fruit appearance, and sugar content. For instance, the dry substances in soft cultivars are nearly 80% of invert sugars (mixture of equivalent extent of fructose and glucose) with high moisture (>30%) and soft flesh. The second group is the semidry cultivars that maintain about 40% invert sugars and 40% sucrose with firm flesh and fairly low moisture (20–30%), This group is the top exporter due to excellent the taste and good shelf- life. The dry cultivars are distinguished by having around 20–40% invert sugars and 40–60% sucrose with hard or dry flesh and low moisture (<20). Cultivars are also considered as early, mid-season, and late cultivars on the basis of duration of time required to mature fruits.

Panga (2014) reported that date palm has 36 chromosomes ($n = 18$; $2n = 36$), but polyploidy cases are also reported with some Iraqi date palm cultivars ($n = 64$). In other cultivars chromosome number differed ($2n = 32, 36$) depending on early or late maturing type. Aneuploidy and euploidy were also reported. The climatic conditions (air temperature and humidity) and soil type play significant role in fruit quality during ripening. Cultivar Deglet Noor develops black nose (blackening and shriveling of the tip) and fruit checks (small, linear scars near the apex) when grown in humid conditions. ‘Deglet Noor’ is a native cultivar of Algeria and Tunisia where it performs as a dry cultivar, while it is generally a semidry cultivar under USA environmental conditions (Krueger 2015). It is noticed that the soft cultivar of Egypt ‘Samany’ showed semidry fruit quality when cultivated in the date palm repository in USA. Moreover, under non-appropriate environmental conditions, cv. ‘Deglet Noor’ yields and quality were often unsatisfactory as in case when cultivated in ‘Punjab’ Province in Pakistan, ‘Wadi an Natrun’ in Egypt. Sometimes these differences occur even in small intra-zones, e.g. hilly or mountain range has low moisture and that is helpful in the longer shelf-life of date fruits. Brac de la Perrière and Benkhalifa (1989) also found some intra-cultivar variability due to environmental effects on fruits of cv. ‘Deglet Noor’ in Algeria and observed that the fruits from Tolga or Biskra oasis are excellent in quality, i.e., semi dry with 20–30% moisture content, buttery, and shiny as compared to the same cultivar grown in M’zab region which were largely drier and smaller and thus of less quality. Abul-Soad et al. (2017) reviewed that the date cultivars around well known date producing countries evaluated so far are about 450 in Saudi Arabia, 400 in Iran, 400 in Iraq, 1000 in Algeria, 250 in Tunisia, 244, 453 in Morocco, 95 in Libya 400 in Sudan, 250 in Oman; 321 in Yemen, 52 cultivars in Egypt 300 in Pakistan, beside numerous cultivars in other dates producing countries. Each dates growing country has its own won cultivars in addition to various other cultivars and their distribution is restricted to these regions due to numerous reasons.

RESULTS AND DISCUSSIONS

Conservation of Date Palm

Ex Situ Conservation

When conservation of plant genetic resources attempted to perform outside or away from their natural habitat, it is termed as ex situ conservation. It can be done by seed and DNA storage, gene banks, collection farms, in vitro preservation or cryopreservation, and botanical gardens (Bekheet and Taha 2013). There are some limited efforts have been made in date palm ex situ conservation that can lead to preserve date palm germplasm for the purposes of successful propagation and improvement programs.

Seed Bank

The seed bank conservation is one of the most widespread and valuable ex situ conservation approach maintaining seed viability at low temperatures and by desiccation. As compared to the 'orthodox' seeds which can be stored for longer durations at subzero temperatures, date palm seeds being 'Recalcitrant' and heterozygous nature cannot be stored for the purpose of conserving genetic resources (Bekheet 2011). Stored date palm seeds in the seed banks can be germinated in in vitro condition. These seedlings could be maintained in vitro at low temperature in order to slow down the growth. Date palm seedlings are widely grown for date palm production with high production.

Svalbard Global Seed Vault

The Svalbard Global Seed Vault, also called Doom's day vault, is a secure seed bank on the Norwegian island of Spitsbergen near Longyearbyen in the remote Arctic Svalbard archipelago, about 1,300 km (810 mi) from the North Pole. The seed vault is an attempt to ensure and provide safety net against accidental loss of genetic diversity in other gene banks during large-scale regional or global crises or loss of samples due to mismanagement, accident, equipment failures, funding cuts, and natural disasters. These events occur with some regularity. War and civil strife have a history of destroying some gene banks. This is a backup for the world's 1,750 seed banks, storehouses of agricultural biodiversity. The seed vault functions like a safe deposit box in a bank. The bank owns the building and the depositor owns the contents of his or her box. A seed sample consists of around 500 seeds sealed in an airtight aluminum bag, and the facility has a storage capacity of 4.5 million seed samples. Date palm seeds could be stored in this international gene bank.

Community Seed Bank

The community seed banks are common at the village level for the preservation of local cultivars and agriculture production in many developing countries (Jain, 2011a). Farmers rely on informal seed systems based on local growers' retention of seed from previous harvests, storage, treatment and exchange of this seeds within and between the communities (Jain, 2011b). The informal seed sector is typically based on indigenous structures for information flow and exchange of seeds. Seed banks managed within this local seed system operate on a small scale at the community level. Community Seed Banks are cost effective with limited resources and facilities. In date palm also the local high quality genetic material is conserved at the village or community level by preserving seeds. For more see http://www.bioversityinternational.org

***In Vitro* Conservation / Repository**

In vitro conservation or in vitro gene bank of the plant genetic resources, various tissues are used such as shoot tips, axillary buds, embryos, callus, and cell suspension cultures (Singh 2009) (Fig.5). They are easy to maintain, less expensive and effective way of storing the plant genetic resources particularly the dioecious nature plants like date palm. In vitro conservation basically involves two stages: at first the in vitro culture establishment and secondly in vitro storage. The in vitro cultures can be conserved for short time (less than one year) or for years to loss some of the viability after sawing once again because of freezing damage. (a) Slow growth or Cold storage-in vitro cultures Slow growth methods are used to conserve plant cultures for relatively longtime storage (few months) by reducing the growth parameters either the temperature and light intensity, adding growth inhibitors, reducing O2 concentration, modifying the nutrient medium which includes dilution of mineral elements, reducing sugar concentrations, and by changing the use of growth regulators, choosing small explants, adding chemicals with osmotic properties (Lédo et al. 2014). Depending on the plant species, slow growth technique allows cultures to be held for 1–15 years under tissue culture regimes with periodic sub-culturing (Jain 2011a, b). However, the high costs of labor and the potential risks of somaclonal variation for some species are the major problems (Cruz-Cruz et al. 2013). There are certain limiting factors of this technique, i.e., reducing temperature cannot be handled effectively when tropical plant species are concerned due to their higher temperature growth habit. Not all types of explants were tested in date palm. Shoot tip explants and callus cultures were successfully employed through slow growth conservation of cv. 'Zaghloul' for 12 months at 5 °C in the darkness (Bekheet et al. 2001). Callus explants of cv. 'Gundila' were also successfully applied for slow growth conservation for the period of 6 and 12 months. The modified medium contained 0.3 M of different sugars with the recovery of 90.73% after four weeks of thawing in normal conditions (Zaid et al. 2011). Incubation temperature during slow growth conservation is reduced from 27 to 15 °C for callus cultures (El-Dawayati et al. 2012). However, it becomes necessary to get high survival rate over 90% and prevent loss of germinated embryos from the conserved embryogenic callus (El-Ashry et al. 2013). However, slowing the growth of callus culture under appropriate conditions could allow enough time for somatic embryo maturation. Subsequently, it will increase the developed somatic embryos. In addition, stored callus culture could serve as stock for micropropagation as per need. In vitro cultures, maintained in slow growth medium, can easily be transported safely. It is worth to mention that the in vitro cultures of date palm are very sensitive for endogenous bacterial contamination. At any time, it may appear when the growth condition is inappropriate.

Cryopreservation

Reed (2017) the cryopreserved collections are highly valuable for the future plant breeding programs and ecosystem restoration. They provide important back up collections for vegetative propagated plants and those with small natural populations or those threatened by human development, environmental changes or development of new diseases. Cryopreserved collections provide long-term security for the plant genetic resources of all types. They provide a secure backup for field collections, insure that little used but unique genotypes are preserved, and store research material worldwide otherwise that would be discarded, save important disease resistance genes and save genes combating future problems facing food and agriculture. Cryopreservation should be considered as Food and nutrition security for the future agriculture. Cryopreservation involves maintaining of living cells and tissue organs at ultralow temperature or in liquid nitrogen (between −79 and −196 °C) for longer periods by halting all the metabolic activities and cell division (Fig.6).

Thus, cells will not undergo genetic changes or somaclonal variations during storage as compared to serial sub-culturing where the cultures are exposed to the risks of contamination and handling errors (Cruz-Cruz et al. 2013). Plant regeneration from cryo-preserved material, e.g. embryogenic cells or somatic embryos is necessary otherwise this approach has no value. There are two modes of cryopreservation protocols based on their physical mechanisms. The classical cryopreservation technique is performed in the presence of ice or ice formation, while the vitrification usually does not involve the ice formation. Since date palm is a dioecious plant, conservation of its genetic resources, using cryopreservation is the best solution being cost-effective and requires small space with the capacity to store large genetic resources without the fear of natural disasters, disease outbreaks, etc. Depending on the plant species and type of cultivars, the cryopreservation technique involves several steps such as selection of the plant material preferably young rapidly growing material, which show resistance against freezing due to smaller size, fewer or small number of vacuoles, and dense cytoplasm; pretreatment of explants in a medium containing osmotically active compounds for dehydration of the tissues and protection of cell membranes; freezing requires to avoid injuries through ice crystal formation); storing at a freezing point where the metabolism activity is suppresses; thawing is done to prevent damage of the cells from the intracellular ice crystals; and post-cryo-treatment minimizes the toxic effect of cryo-protectants and reduce the osmotic shock. Abul-Soad, et al (2017) reported testing of several date palm explants to cryo-store using caulogenic meristem, friable callus, pro-embryogenic masses, somatic embryos, shoot apices, and pollen. Bekheet et al. (2007) cryopreserved nodular callus of date palm initially at 0 °C for 2 h and then transferred into liquid nitrogen (−196 °C) for 48 h. The recovery percentage after thawing was 80% on 1 M sucrose-pre-culture medium. Fki et al. (2011, 2013), the Tunisian group, tested three cryopreservation vitrification protocols -, standard (tube) droplet, and encapsulation- were tested.' The standard vitrification gave the highest recovery rates using small explants (2 mm), while the larger explants (>3 mm) died after thawing stage. Salma et al (2014) cryopreserved poly-embryonic masses (PEMs) using droplet-vitrification and dehydration cryo-plate techniques. The recovery percentages of pro-embryos or PEM of cultivar was highly dependent on genotype after transfer to the standard culture medium containing 3.3 M glycerol + 2.4 M ethylene glycol + 0.4 M sucrose + 1.9 M dimethyl sulfoxide. Bekheet (2015) studied the effect of salt mixture (NaCl, MgCl, and CaCl2) along with other osmotic stimulators such as mannitol and polyethylene glycol (PEG) for cryopreservation of embryogenic cultures of date palm. The highest values of fresh mass were at salt tolerance ratio of 1500 ppm.

Cryopreservation of date palm germplasm is still at the infancy stage in terms of plant regeneration from cryo-stored explants. This technique has been tried mainly in Egypt and Tunisia, and the results have been published. In vitro date palm culture is highly genotypic dependent for high efficiency of plant regeneration, and that why limited number of date cultivars has been tested for somatic embryogenesis and other tissue culture activities. Genetic fidelity of plant regenerated from cryo-stored material must be determined by using molecular markers, just to confirm genetic stability of regenerated plants.

In Situ Conservation

In situ as the term indicates literally mean 'in place,' involving conservation of plants in its natural habitat to which it is adapted and maintained by farmers within the traditional agricultural systems and allows the recovery of germplasm in their natural surroundings (Singh 2009; Rao and Sthapit 2012). Date palm growers are playing an important role in preserving the biodiversity of traditional date palm grove and gardens by continuous use of century-old practices in maintaining the traditional date cultivars and propagation of the newly developed races with distinctive properties. Since the conservation of such agro-biodiversity is carrying out as on-farm, therefore such type of conservation is also termed as on-farm conservation. In such a way, the genetic diversity of target species is managed and wild plants have been maintained within the traditional agricultural or horticultural systems. It has helped the species to adapt gradually with new variations in the gene pool caused by environmental conditions such as global warming, changed rainfall patterns (Heywood and Dulloo 2005). Sustainable on-farm and in situ conservation of date palm diversity is only promising when farmers, academia, and government organizations show interest in recognizing the benefits in terms of genetic, economic, social, and environmental point of view and by implementing the private utility benefits to the individual grower or user. On-farm conservation is encouraging in several Middle East and North African countries as a potential method of date palm conservation strategy. When farmers get motivation from the state, they also show their interest in this global cause (Jaradat 2015). Nowadays, in date-producing countries, the major crops growing concern is to exchange the information to develop the date palm sector. The great advance and wide usage of social media programs made groups with direct contact able to exchange the photographs and movies instantly either on national or on international levels. This is expected to not only encourage the on-farm conservation but also support all other activities in date palm. The status of on-farm conservation of date palm is still limited. However, there are numerous small-scale conservations or rather germplasm collection stations or farms maintaining the local cultivars in more or less all date-producing countries. This could keep the progeny of elite landraces and commercial cultivars of a limited population at same place and prevent losing such valuable genetic resources. It is a practice for the date palm growers in the non-systematic farms to regularly clean their orchards by detaching the offshoots from parent female productive trees and plant them once again in between the adult trees or establish a new orchard.

Field gene bank

Field gene bank is one the ways to collect, maintain and conserve the date palm genetic resources by vegetative propagation for maintaining their genetic makeup true to type for the long-term preservation of the genetic or inter-specific variability. This approach for germplasm conservation is always risky of damage by natural disasters, pest and pathogen problems (Singh 2009), and relatively expensive and requires huge space. However it is providing easy and ready access to conserve palms for research and their utilization. There are number of field gene banks in almost all date-producing countries including for example King Faisal University, in southeastern Al-Hassa (Saudi Arabia), comprising 31 Saudi Arabian cultivars collected from 7 major growing regions and 26 exotic cultivars (Al-Ghamdi 2001; Aleid et al. 2015). A project started by General Board of Date Palm (GBDP) with the help of the ministry of Agriculture in Iraq has collected 497 cultivars in various regions of Iraq (Khierallah et al. 2015). The Kuwait Institute for Scientific Research (KISR) at Kuwait university main campus maintains 34 female and 6 male cultivars (Sudhersan et al. 2015) (Fig.7). The establishment of such collective farms is helpful in evaluating and comparing the fruit quality of alien cultivars at the experimental stations, and in making growers to take the right decisions to introduce valuable alien cultivars that suit the local environment.

CONCLUSIONS

The collected elite germplasm are highly valuable for the future plant breeding programs and ecosystem restoration. They provide important back up collections for plants, which are in danger of losing due to human development, environmental changes or development of new diseases. All collections from different means of conservation provide long-term security for the plant genetic resources of all types. These collections provide a secure backup for field collections and save important abiotic and biotic resistance genes and save genes combating future problems facing food and agriculture. Climate changes endanger the economic migration. Climatic factors such as extreme floods, drought, cyclones, erosion and land degradation make natural resource-based livelihoods more tenuous, force inhabitants to migrate for better living. In vitro conservation has several distinct advantages, e.g. the material can be maintained in a pathogen-free state facilitating safer distribution without going through quarantine. Furthermore, the cultures are maintained under the controlled growing conditions without subjected to any environmental disturbances. Cryopreservation is a long-term in vitro storage of genetic material, however needs a reliable plant regeneration system from cryo-stored material without showing any genetic variability. The regenerated plants Date palm conservation is an excellent system for Food and nutrition security for the future sustainable production facing climatic changes and genetic erosion. Together with the air we breathe and the water we drink, crop diversity and conservation are most fundamentally important resources for human life on earth.

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REFERENCES

Ahuja, M.R. 2017. Climate Change, Genetic Diversity, and Conservation of Paleoendemic Redwoods. pp 69-94. In M.R. Ahuja, and S. M. Jain (eds.), Biodiversity and Conservation of Woody Plants, Springer, The Netherland.

Abul-Soad, A.A., Jain, S.M., and Mushtaque Jatoi, A. 2017. Biodiversity and Conservation of Date Palm. pp 313-352. In: In M.R. Ahuja, and S. M. Jain (eds.), Biodiversity and Conservation of Woody Plants, Springer, The Netherland.

Al-Ghamdi, A.S. 2001. Date palm (*Phoenix dactylifera* L.) germplasm bank in King Faisal University, Saudi Arabia. Survival and adaptability of tissue cultured plantlets. Acta Hort. 450:241–244.

Aleid, S.M., Al-Khayri, J.M., and Al-Bahrany, A. M. 2015. Date palm status and perspective in Saudi Arabia. pp 125–168. In: J.M. Al-Khayri, S.M. Jain, and D.V. Johnson (eds.), Date palm genetic resources and utilization- Asia and Europe, Vol.2. Springer, Netherlands.

Alhudaib, K., Arocha, Y., Wilson, M., and Jones, P. 2007. Al-Wijam, a new phytoplasma disease of date palm in Saudi Arabia. Bull. Insectology 60(2):285-286.

Bekheet, S.A. 2011. In vitro conservation of date palm germplasm. pp 337–360. In: J.M. Al-Khayri, S.M. Jain, and D.V. Johnson (eds.), Date palm biotechnology. Springer, Netherlands.

Bekheet, S.A. 2015. Effect of cryopreservation on salt and drought tolerance of date palm cultured in vitro. Sci. Agr. 9:142–149.

Bekheet, S.A., Taha, H.S., and Saker, M.M. 2001. In vitro long-term storage of date palm. Biol. Plant. 45:121–124.

Bekheet, S.A., Taha, H.S., Saker, M.M., and Solliman, M.E. 2007. Application of cryopreservation technique for in vitro grown date palm (*Phoenix dactylifera* L.) cultures. J. Appl. Sci. Res. 3:859–866.

Bekheet, S.A., and Taha, H.S. 2013. Complementary strategy for conservation of date palm germplasm. Glob. J. Biodivers. Sci. Manag. 3:96–107.

Brac de la Perrière, R.A., and Benkhalifa, A. 1989. Identification de cultivars de dattiers (*Phoenix dactylifera* L.) du sud-ouestalgérien. Plant Gen. Res. Newsl FAO/IBPGR.78/79:13–20.

Cruz-Cruz, C.A., González-Arnao, M.T., and Engelmann, F. 2013. Biotechnology and conservation of plant biodiversity. Resources 2:73–95

El-Hadrami, A, Daayf, F., Elshibli, S., Jain, S.M., and El-Hadrami, I. 2011. pp 183-203. Somaclonal variation in date palm. In: J.M. Al-Khayri, S.M. Jain, and D.V. Johnson (eds.), Date palm biotechnology. Springer, Netherlands.

El-Ashry, A.A., Shaltout, A.D., El-Bahr, M.K., Abd EL-Hamid, A., and Bekheet, S.A. 2013. In vitro preservation of embryogenic callus cultures of two Egyptian dry date palm cultivars at darkness and low temperature conditions. J. Hort. Sci. Ornament Plants 5:118–126

Fki, L., Bouaziz, N., Sahnoun, N., Swennen, R., Drira, N., and Panis, B. 2011. Palm cryobanking. CryoLetters 32:451–462.

Fki, L,, Bouaziz, N., Chkir, O., Benjemaa-Masmoudi, R., Rival, A., Swennen, R., Drira, N., and Panis, B. 2013 Cold hardening and sucrose treatment improve cryopreservation of date palm meristems. Biol. Planta. 57:375–379

Heywood, V. H., and Dulloo, M.E. 2005. In situ conservation of wild plant species, a critical global review of good practices. International Plant Genetic Resources Institute (IPGRI) Technical Bulletin 11, Rome, Italy

Jain, S.M. 2011a. Date palm genetic diversity conservation of for sustainable production. Acta Hort. 882:785-791.

Jain, S.M. 2011b. Prospects of in vitro conservation of date palm genetic diversity for sustainable production. Emir. J. Food Agric. 23 (2): 110-119.

Jaradat, A.A. 2015. Genetic erosion of *Phoenix dactylifera* L.: perceptible, probable, or possible. pp 131–213. In: M.R. Ahuja and S.M. Jain eds.), Genetic diversity and erosion in plants, Springer, Netherlands.

Johnson, D.V. 2011. Introduction: date palm biotechnology from theory to practice. Date Palm Biotechnol. 1–11.

Khierallah, H.S., Bader, S.M., Ibrahim, K.M., and Al-Jboory, I.J. 2015. Date palm status and perspective in Iraq. pp 97–152. In: J.M. Al-Khayri, S.M. Jain and D.V. Johnson (eds.) Date palm genetic resources and utilization, Vol.2. Asia and Europe. Springer, Netherlands.

Krueger, RR (2015) Date Palm Status and Perspective in the United States. In Al-Khayri JM, Jain SM, Johnson DV (Eds) Date Palm Genetic Resources and Utilization. Volume 1: Africa and the Americas. Springer Netherlands, pp 447-485

Lédo, A.D.S., Moura, C.R.F., Machado, C.D.A., Ramos, S.R.R., Silva, A.V.C., and Lédo, C.A.D. 2014. Mannitol for coconut ex situ conservation by minimum growth. Pesquisa Agropecuária Bras 49:148–151.

Panga, J.A. 2014. Plants of AMS Garden: A Garden in the Arabian Deserts of Dubai. Xlibris Corporation

Rao, V.R., and Sthapit, B. R. 2012. Tropical fruit tree genetic resources: status and effect of climate change. pp 97–137. In: B. R. Sthapit, V.R. Rao, and S.R. Sthapit (eds.). Tropical fruit tree species and climate change. Bioversity International, New Delhi, India

Reed, B.M. 2017. Plant cryopreservation: a continuing requirement for food and ecosystem security. In vitro 53: 285-.288.

Salma, M, Fki, L, Engelmann-Sylvestre, I, Niino, T, and Engelmann, F. 2014. Comparison of droplet-vitrification and D-cryoplate for cryopreservation of date palm (*Phoenix dactylifera* L.) polyembryonic masses. Sci. Hort. 79:91–97.

Shabani, F., Kumar, L., and Taylor, S. 2012. Climate Change Impacts on the Future Distribution of Date Palms: A Modeling Exercise Using CLIMEX. PLoS ONE 7(10): e48021. doi:10.1371/journal.pone.0048021

Sedra, M.H. 2011. Development of new Moroccan selected date palm varieties resistant to bayoud and of good fruit quality. pp 513-532. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.). Date Palm Biotechnology, Springer, Dordrecht.

Singh, B.P. 2009. Germplasm introduction, exchange, collection/evaluation and conservation of medicinal and aromatic plants-their export potential. pp 8–11. In: P.C. Trivedi (ed.) Medicinal plants: utilization and conservation. Aavishkar Publishers, Jaipur, India.

Sudhersan, C., Sudhersan, J., Ashkanani, J., and Al-Sabah, L. 2015. Date palm status and perspective in Kuwait. pp 299–321. In: J.M. Al-Khayri, S.M. Jain, and D.V. Johnson (eds.) Date palm genetic resources and utilization, Vol. 2, Asia and Europe. Springer, The Netherlands.

Zaid, A., and de Wet, P.F. 2002. Pollination and bunch management. pp 227–269. In: A. Zaid (ed.) Date palm cultivation. FAO Plant Prod. & Prot. Paper 156 Rev, Vol 1,

Zaid, Z. E., El-Dawayati, M.M., Baker, E.I., and Gomaa, A.H. 2011. Studies on storage under minimal growth condition of date palm callus explants. Proc. Arab palm conference, National Centre for Agricultural Technologies, King Abdul Aziz City for Science and Technology, Riyadh, Saudi Arabia, p 401–420.

Figures

Figure 1: Date palm-tree of life, https://commons.wikimedia.org/w/index.php?curid=133553

Fig.2 Melting glacier due to climate change.

Fig. 3 Genetic diversity in date palm, clearly showing variation in fruit color and shape

Photo By Miksu - Own work; https://commons.wikimedia.org/w/index.php?curid=61440471
Fig, 4. Svalbard Global Seed Vault, Norway

Figure 5: In vitro conservation of germplasm

Figure 6: Basic equipment for cryopreservation

Fig.7: Date palm germplasm collection at The Kuwait Institute for Scientific Research (KISR) at Kuwait university main campus

Production of single cell protein from some date by-Products

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Abstract

The single cell protein produced has a good source of essential amino acids. This study aimed to utilize some date by-products such as date flesh to produce single cell protein. The flesh of dates from unknown cultivars and three varieties are used to produce a single cell protein by *Saccharomyces cerevisiae* ATCC64712 under different conditions (different sugar concentrations in the extract media, different temperature and different pH). The results showed that the optimum production of the biomass (42.85g/l) was produced from date extracts media at 18% sugar, 30˚C and pH values 4.5. To reduce the nucleic acid content in the biomass, heat shock was used. The effect of heat shock on protein content and nucleic acids percentage for studied yeast strain was 75.83%, and the maximum reduction of nucleic acids was observed at pH4 and 60°C for 30 sec with 20.88% loss of its protein content. The present study provides evidence that the date by-product is a great source for single cell protein that can be used in variable fermentations.

Keywords: Single cell protein, date by-products, *Saccharomyces cerevisiae* ATCC64712, amino acids.

INTRODUCTION

The date palm (Phoenix dactylifera L.) is one of the most economically important Fruit tree grown in Egypt. Production of Egypt alone represent about 20% of the total world production at 2012 (Bekheet, 2013 and FAO, 2012). Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrates content (70 – 80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Myhara et al. 1999; Al-Farsi et al.2005 and Mrabetet al. 2008). used as a coffee substitute) has also been introduced recently to the market (Rahman et al. 2007). Date seed oil has been used to replace the portions of other vegetable oils in body creams, shampoos, and shaving soap formulations, and, in general, the quality of these cosmetic formulations is encouraging (Devshonyet al.,1992)

The aim of this study was to determine the nutritional value, proximate analyses, fatty acid composition, sugar contents and bioactive properties of Siwi date seeds provided from Kharja Date Packing Factory.

MATERIALS AND METHODS

Materials

Date samples: This study was carried out of six date by-products fruit which lies in two divisions, classified and un classified (Manthour or seedling). Date varieties (classified ones) include; Hayany and Zaghloul which are soft dates and Saidy which is semi dry date. The unclassified (Manthour, Mt.) date fruits called Mt.1, Mt.2 and Mt.3. All these date fruits were obtained from Qena Governorate Kharja Date Packing Factory, Kharja oasis, The New Valley Governorate during the 2013 seasons.

Yeast strain

Saccharomyces cerevisiae EMCC ATCC64712 obtained from Microbiological Resources Center, Cairo MIRCEN, Ain Shams University, Egypt.

Media used for yeast maintenance:

Glucose peptone yeast extract medium was used for yeast maintenance composition is follows DSM Medium 186:

Yeast extracts	3.0g
Peptone from soybeans	5.0g
Malt extracts	3.0g
Glucose	10.0g
Agar	15.0g
Distilled water	1000ml

Single cell protein production:

Preparation of date juice for single cell protein production:

The mixture of (Six date by-products) date fruits was cleaned from extraneous materials then washed and the pits were removed. The date flesh cutted in to small pieces to further decrease their size in order to improve the extraction efficiency .The small pieces of flesh were mixed with water (1:4 flesh: water, w/v) and soaking for 12 hr under cooling after that boiled gently for 30 min, then blended in an electric mixer (Blinder) for one minute and filtered through double fold cheese cloth. date fruit juice was passed through double chess cloth to exclude all large undigested particles. Date juice was packaged in double polyethylene bags and kept at -15˚C till further experiments. The date fruit juice was prepared from date mixture as illustrated in Fig(1)

Effect of some environmental factors:

The most environmental conditions were studied to produce the highest biomass and protein content from the tested yeast strain.

pH values. Date juice medium was adjusted at different pH values ranged from 3.5 to 6.0 pH. Flasks of different pH levels were incubated at 30˚C for 96 hr on rotary shaker at 150rpm.

Temperature. Flasks containing date juice media with 18% sugar content were adjusted to 4.5 pH and incubated at different temperatures; 25, 30, 35 and 40˚C for 96 hr using rotary shaker at 150rpm.

Inoculum size. Suspension of cell obtained from yeast strain was used as inoculum size in a range between 1-6 % (0.5 to 3.0ml) inoculum. The inoculated flasks contained 50ml media were incubated at 30˚C on rotator 150 rpm for 48 hr.

Biomass production using fermentor incubation period at optimal condition

Yeast strain incubated in date juice medium three liter in fermentor at aeration rate 2.5 1/ min, pH 4.5 and 30˚C for 96 hr. Biomass, protein content and TCP were determined.

Determination of cell dry weight:

The yeast growth was harvested by centrifugation at 3000 rpm for 15 min. The supernatant was then decanted and the residual cell suspended in distilled water and recentrifugated. This was repeated twice and finally the cells were then transferred to measuring flask (100) using distilled water. Aliquots of 5ml were then taken and dried at 105˚C overnight and results were given as g dried yeast per liter of the used medium (White,1954)

Studying the obtained yeast biomass: the biomass of yeast strain was centrifuged, washed twice with distilled water it was dried at 70˚C till constant weight was obtained then the following studies were carried out on them.

Total soluble solids (TSS):

Total soluble-solids values as Brix were determined with Digital Refract meter (Model no. RX 5000, ATAGO, Japan) at 25˚C.

The pH value:

The pH values were measured using digital pH meter model no.APX 175, Control Dynamics Ltd., Bangalore, India.

Crude protein and amino acids:

The nitrogen content was analyzed using the standard Kjeldahl procedure (AOAC, 2000). Protein content was determined by multiplying the nitrogen content by 6.25 according to Merrill and Watt (1973). While amino acids were determined using Automatic Amino Acid Analyzer (AAA 400INGOS Ltd). Hydrolysis of total amino acids. Acid hydrolysis was carried out according to the method of Csomos and Simon-Sarkadi (2002). Free amino acids extraction was carried out according to the method of Shalabia (2011).

RESULTS AND DISCUSSION

Production of single cell protein (SCP):

Environmental conditions:

The most important variables affecting the cultivation and production of SCP are the environmental conditions under which the microorganisms were cultivated (incubation temperature, pH value, incubation period, inoculums size and composition of the growth medium.

Effect of pH value:

Extracts of date fruits containing 18 % sugar, adjusted to different pH values such as 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 were used to produce single cell protein by the selected yeast. Results in Table (1) showed that the slightly acidic pH (3.5–6.0) was appropriate for biomass production by the S. cerevisiae ATCC 64712.The maxima of biomass, crude protein (CP) and total crude protein (TCP) were obtained at pH 4.5 followed by pH 5.0. The maxima production of biomass (42.85 g/l), crude protein CP (67.8%) and total crud protein TCP (29.05g/l) were obtained at pH 4.5 followed by 32.45 g/l, 60.18% and 19.52g/l, respectively at pH 5.0. Our finding is supported by the suggestion that a weak acidic medium is more appropriate for the overall growth of yeasts (Pramanik, 2003). The results are agreeing with those obtained by Abou-Aly (1996) and Hassan (2012). They found that the biomass and total crud protein were gradually increased by increasing pH up to 4.5. However, other observations suggest that the pH range of any yeast strain could vary depending on the medium composition. In this context, Onishi (1963) showed that the pH range for the growth of Z. Rouxii strain isolated from the soy sauce process without NaCl is very broad (pH 3.0–7.0), while in a medium containing 18 % NaCl, the pH range for growth is narrow (pH 4.0–5.0).

Data of statistical analysis of biomass, crude protein and total crude protein are shown in Table (1). Data provided that there were high significant differences among biomass, crude protein and total crude protein produced by yeast strain at pH 4.5 and at the other pH values with the same conditions. On the other hand, data also revealed that there were no significant differences in among biomass, crude protein and total crude protein production at pH4, pH5 and pH5.5.

Effect of incubation temperature:

Samples of date extracts were adjusted to 20 Brix (18 % sugar concentration) and pH 4.5, then inoculated with yeast and maintained for produced single cell protein at different temperatures such as, 25, 30, 35 and 40˚C. The optimum temperature for single cell protein production byS. cerevisiae ATCC 64712after 60 hr with standard inoculum grown in sugar date juice (18%) are shown in Table (18).

Results in table (2) showed that S. cerevisiae ATCC 64712 strain was able to grow up to 40˚C. The maximum yield was 42.85g/L at 30˚Cand decreased to 16.51g/l at 40˚C. These results demonstrated the 30˚C was the optimum degree for biomass production, crude protein and total crude protein.

Based on the information available in the literature, the optimum temperature varies widely among the yeast strains that 28˚C is the most favorable temperature for biomass production by Kluyveromyces lactis grown on whey permeate. Lee et al. (1993) reported that the optimum temperature for thermo tolerant Candida tropicalis used for SCP production was 38 °C. Rajoka et al. (2006) studied the production of SCP by Candida utilis at different temperatures (20-45˚C) in a stirred fermentor and reported that the maximum production of crude protein was realised when the fermentation temperature was maintained at 35˚C. They also found that the production of crude protein decreased above 35˚C. High temperature can cause inactivation of enzymes of the metabolic pathway, while low temperature may not permit the flow of nutrients across the cell membrane, resulting in a high demand for maintenance energy. However, at low temperature, the enzyme activities are expectedly low (Roels, 1983 and Converti and Dominguez, 2001).

Data of statistical analysis of biomass, crude protein and total crude protein data provided that there were high significant difference among biomass, crude protein and total crude protein produced by yeast strain at 30˚Cand other produced at different temperature used except in between 25˚C and 35˚C, there was no significant difference in biomass produced by yeast.

Effect of inoculum size:

Samples of date extracts were adjusted to 20 20 Brix (18% sugar concentration),4.5 pH, 30˚C temperatures and inoculated with yeast for Suspension of yeast cells obtained from active slants prepared to study the effect of inoculum size on biomass production which were used in range between 1-6% (0.5-3.0 ml inoculum medium).

Data in Table (19) showed that the biomass, crude protein and total crude protein gradually increased with the inoculum size increasing to the maximum 42.85 g/l, 67.8% and 29.05g/l at the inoculum 5%, respectively. These results are agreement with Alian et al. (1990), Francisco et al. (2010) and Hassan (2012). They reported that the best ratio for production of single cell protein for Saccharomyces. cerevisiae was 5% v/v of inoculum size.

Data of statistical analysis of biomass, crude protein and total crude protein from these it was clear that there were high significant differences among biomass and total crude protein produced by yeast strain at all inoculum except in between1.5 and 3 inoculum size. On the other hand, data also revealed that there was no significant difference in between 1.0 and 3.0 inoculum size in crude protein production. acids.

Effect of sugar concentration:

The suitability of date extracts containing different sugar concentrations to produced single cell protein was studied by S. cerevisiae ATCC 64712. The different tested concentrations of sugar in date extract were 10 % (12 Brix), 12 % (14 Brix), 14 % (16 Brix), 20 % (18 Brix), 22% (24 Brix) and 24 % (26 Brix). The effect of different sugar concentration on the biomass, protein and total crude protein of S. cerevisiae ATCC 64712 was studied.

Results in Table (4) illustrated that concentration of the date juice significantly affected the productivity of biomass and crude protein of the yeast. The biomass and crude protein increased with the increase in concentration of the substrate up to 18 %. The maximum biomass and crude protein were recorded when they were grown in 20% of date juice after 60 hr at 30˚C and 4.5 pH. When the concentration of the substrate increased up to 20%, the biomass and crude protein of the yeast strains decreased by 20.91 and 16.62%, respectively. These results proved that 20 % of date syrup (18% sugar) is the most appropriate concentration to encourage the growth and production of the biomass by the yeast strain. They seemed to carry out all normal physiological processes in a moderate concentration of sugars, while the increase in date juice concentration slowed down their growth. The decrease in growth rate in high concentrations of date juice could be attributed to the viscosity of the medium and plasmolysis of yeast cells that retard or stop their growth (Pramanik, 2003).

In similar studies, Hashem et al. (2014) optimised the cultural conditions for production of single-cell protein by Yeast strains (Zygosaccharomyces rouxii KKUY-0157 and Hanseniaspora uvarum KKUY-0084) spoilage date juice (SDJ). They showed the best growth and production of biomass at 25 ˚C in a 20 % date juice concentration, they could resist an increase in temperature to 30 ˚C, and they could grow in higher concentrations of date juice. They noticed that the growth and biomass productivity of the two strains were greatly enhanced by adding metals such as Mn or Mg as well as a nitrogen source (tryptone).

Data given in Table (4) indicated that there was high significant differences in the biomass production in all sugar concentration were used except inbetween16,22 and 24% sugar. Also there were high significant differences in crude protein between all sugar concentration used. Besides, there was high significant difference in the total crude protein except inbetween 22 and 24% sugar concentration were used.

REFERENCES

Abou-Aly, H. E. (1996): Microbiological studies on protein production by microorganism, Ph.D. Thesis, Dept. of Agric. Botany Fac. Of Agric., Moshtohor, Zagazig Univ., (Benha Branch).

Al-Farsi, A. M. & Lee, C. Y. (2007): Optimization of phenolics and dietary fiber extraction from date seeds. Food Chem., 108: 977985.

Al-Farsi, A. M.; Alsalvar, C.; Morris, A.; Baron, M. & Shahidi, F. (2005): Compositional and sensory characteristics of three native sun-dried date *(Phoenix dactylifera L.)* varieties grown in Oman. J. Agric. Food Chem., 53, 7586-7591.

Al-Farsi, A. M.; Alsalvar, C.; Morris, A.; Baron, M. & Shahidi, F. (2005): Compositional and sensory characteristics of three native sun-dried date *(Phoenix dactylifera L.)* varieties grown in Oman. J. Agric. Food Chem., 53, 7586-7591.

Al-Farsi, M.; Alasalvar, C.; Al-Abid, M.; Al-Shoaily, K.; Al-Amry, M. &Al-Rawahy, F. (2008): Compositional and functional characteristics of dates, syrups and their by-products. Food Chem., 104: 943-94.

AlFarsi, M.; Alasalvar, C.; AlAbid, M.; AlShoaily, K.; AlAmry, M. & AlRawahy, F. (2007): Compositional and functional characteristics of dates, syrups and their byproducts. Food Chem., 104 (3): 943947.

AlFarsi, M.A. & Lee, C.Y. (2008): Nutritional and functional properties of dates: a review. Crit. Rev. Food Sci. Nutr., 48:877887.

Alian, A. N.; Hoda, G. El-Masry.; Nagwa, M. El-Shimi & Fadel, M. A. (1990): Utilization of pea peels sugar hydrolyzate in yeast protein production. Annals Agric.Sci., Fac. of Agric., Ain Shams Univ., Cairo, Egypt, 35(1):157-166.

AOAC (2000): Association of Official Analytical Chemists. Official methods of analysis (17th Ed.). Washington., D.C., U.S.A.

Converti, A & Dominguez, J. M.(2001):Influence of temperature and pH on xylitol production from xylose by *Debarryomyces hansenii*. Biotechnol Dioeng, 75:39- 45.

Csomos, E. & Simon-Sarkadi, L. (2002): Characterisation of tokaj wines based on free amino acid and biogenic amine using ion exchange chromatography. Chromatographia Supplement,

Devshony, S.; Eteshola, A. & Shani, A. (1992): Characteristics and some potential application of date palm (*Phoenix dactylifera L.)* seeds and seed oil. J. Am. Oil Chem. Soc., 69: 595597.

FAO (2013): Statistical Databases. http://faostat.fao.org Accessed April 07 2013.

Farncisco, Carrau; Karina, M.; Laura, F.; Eduarado, B & Edurdo, D. (2010): Effect of *Saccharomyces cerevisiae* inoculum size on wine fermentation aroma compounds and its relation with assimilable nitrogen content. Inte. J. Food. Microbiol. 143:81-85.

Hashem, M.; Hesham, A. E.; Alamri, S. A. & Alrumman, S. A. (2014): Production of single-cell protein from wasted date fruits by *Hanseniaspora uvarum KKUY-0084 and Zygo saccharomyces* J. Ann Microbiol, 64:1505-1511.

Hassan, M. A. A. (2012): Evaluation of some yeast strain for single cell protein production (M.Sc). Thesis. Agri. Bot., Dept., Agri., Fac. Benha. Unvi.

Lee,C.; Yamakawa, T; Kodama, T. (1993): Rapid growth of thermo tolerant yeast on palm oil. Biotech., 9:187-190.

Merrill, A. L. & Watt, B. K. (1973): Energy Value of Foods: Basis and derivation. Agric. Handbook No. 74. Washington, DC, ARS United States.

Onishi, H. (1963):Osmophilic yeasts. Adv Food Res 12:5394 Pacheco, M.T.;Caballero-Cordoba, G. M. and Sgarbieri, V.C. (1997):Composition and nutritive value of yeast biomass and yeast protein concentrates. J. Nutr. Sci. Vitaminol 43:610-612.

Pramanik, K. (2003):Parametric studies on batch alcohol fermentation using Saccharomyces yeast extracted from toddy. J. Chin. Inst. Chem., Engrs., 34:487-492.

Rahman, M. S.; Kasapis, S.; Al-Kharusi, N. S. Z.; Al-Marhubi, I. M. & Khan, J. A. (2007): Composition characteristics and thermal transition of date pits powders. J. Food Eng., 80: 110.

Rajoka, M. I.(2004):Influence of various fermentation variables on exoglucanase Production in *Cellulomonas flavigena*. J .ElectronicBiotech.7(3):0717-3458.

Rajoka, M. I., Khan, S. H.; Jabba., M. A. ; Awan, M. S. & Hashmi, A .S. (2006): Kinetics of batch single cell protein production from rice polishing *Candida utilis* in continuously aerated tank reactors. System in yeast. J. Bio-resource Tech., 97: 1934-1941.

Rajoka, M. I.; Latif, F.; Khan, S. & Shahid, R. (2004): Kinetics of -galactosidase by a cycloheximideresistant mutant of *Kluyveromyces marxianus*.J. Biotech.Letters, 26(9):741-746.

Roels, J.A. (1983):Energetics and kinetics in biotechnology. Elsevier,

Shubbar, B. H. (1981): Sugar extraction from dates. Date Palm J., 1(1):61-78.
White, J. (1954): Yeast technology. Champan and Hall, London. (c.f Hammouda,1988).

Tables

Table (1): Effect of initial pH of the medium on biomass production and protein content of *Saccharomy cescerevisiae ATCC 64712*.

initial pH	Biomass g/l	CP %	TCP g/l
3.5	28.07c±0.043	42.00d± 0.15	11.79e±0.30
4.0	31.94b±0.54	58.0b±0.154	18.51c±0.06
4.5	42.85a±0.164	67.8a±0.06	29.05a±0.100
5.0	32.45b±0.259	60.18b±0.748	19.52b±0.089
5.5	32.00b±0.66	59.46b±0.308	19.02bc±0.355
6.0	27.60c±0.398	45.60c±0.396	12.57d±0.087
LSD	1.249	2.347	0.627

Table (2): Effect of incubation temperature on biomass production and protein content of Saccharomycescerevisiae ATCC 64712.

Incubation temperature ˚C	Biomass g/l	CP %	TCP g/l
25	41.36b±0.29	51.71c±0.053	21.29c±0.203
30	42.85a ±0.16	67.8a ±0.05	29.05a ±0.10
35	42.4ab±0.14	62.57b±0.057	26.52b±0.083
40	16.51d±.811	49.6d±0.58	8.17d±0.31
LSD	1.456	0.955	0.636

Table (3): Effect of inoculum size on biomass and protein content of *Saccharomyces cescerevisiae ATCC 64712.*

Inoculum size		Biomass g/l	CP %	TCP g/l
0.5	1%	32.09e±0.57	45.46e±0.57	14.57e±0.07
1.0	2%	35.2d±0.57	54.54d±0.56	19.18d±0.11
1.5	3%	37.60c±0.58	56.54c±0.58	21.24c±0.10
2.0	4%	40.50b±0.57	65.16b±0.58	26.38b±0.32
2.5	5%	42.85a ±0.16	67.8a ±0.05	29.05a ±0.10
3.0	6%	38.70c±0.58	53.73d±0.44	20.78c±0.15
LSD		1.63	1.55	0.52

Table (4): Effect of sugar concentration onbiomass productionand protein content of Saccharomycescescerevisiae ATCC 64712.

Sugar concentration %	Biomass g/l	CP %	TCP g/l
10	22.93f ±0.672	44.3h ±0.057	10.15g ±0.29
12	24.96e±0.49	44.8g±0.058	11.18f±0.20
14	27.49d±0.017	56.32d±0.005	15.47d±0.008
16	31.3c±0.09	64.61b±0.005	20.21b±0.060
18	42.85a±0.16	67.8a±0.05	29.05a±0.10
20	33.62b±0.18	56.53c±0.005	19.00c±0.11
22	31.35c±0.12	46.83e±0.05	14.64e±0.03
24	31.28c±0.16	45.6f±0.008	14.26e±0.07
LSD	0.954	0.107	0.423

Figures

Fig (1): Morphological picture of the studied date samples

Raw date fruits Sorting

Washing

Pits ← Pitting

Water

Soaking (12 hr) under cooling then boiling for 30 min Blending

Filteration

Storage (-15˚C)

Fig (2): Extraction of date juice flow diagram (Shubbar, 1981).

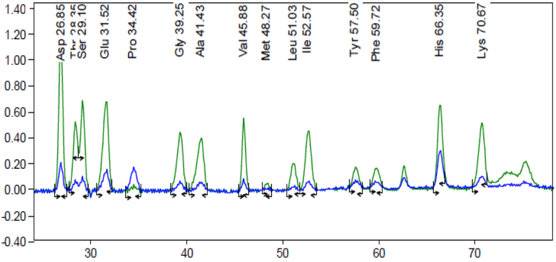

Fig (3): Amino acid content of *Saccharomy. cescerevisiae(ATCC 64712).*

Biotechnological Studies on the Acclimatization of Date Palm Plantlets Produced Via Tissue Culture Techniques. 1- Effect of chemical compounds

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Abstract

Date palm (*Phoenix dactylifera* L.) cv. Gondella has a high quality dates but unfortunately its offshoots of short supply since fungal and bacteria diseases and insect infection uncontrolled. *In vitro* direct embryogenesis formation is considered a good tool for problem recovery and mass propagation. Leaf primordial explants were cultured on MS (Murashige and Skoog 1962) basal medium supplemented with a ascorbic acid (75 mg/L), citric acid (75 mg/L), Polyvinylpyrrolidone (1.5 g/L), NaH2PO4. 2H2O (170 mg/L), activated charcoal (1.5 g/L) and Yeast extracts (1.0 g/L) for 2 weeks. Explants were re-cultured monthly on modified MS medium (MMS) supplemented with 2iP (5.0 mg/L), Kin (0.5 mg/L), BA (0.1 mg/L), NOA (0.25 mg/L), putrescine (50 mg/L), ABA (0.05 mg/L), Glutathione (10 mg/l), asparagen (50 mg/L) a ascorbic acid (75 mg/L), citric acid (75 mg/L), activated charcoal (1.5 g/L) and sucrose (40 g/L) solidified with gelrite (2.0 g/L). Direct embryos were formed on leaf primordial explants cultured on MMS medium after 4 months of incubation under dark/light conditions. MMS medium supplemented with Ca-pantothenate (20 mg/L) affect positively embryos growth characters. Healthy grown shoots individually cultured on basal MS medium supplanted with IBA (0.3 mg/L) , NAA (0.3 mg/L) , spermidine (100 mg/L), sucrose (30 g/L) and solidified with phyto- agar (6.0 g/L), recorded the highest significant values of roots number and roots length after 8 weeks of treatment incubation. A mixture containing residual sugar cane mud and perlite (1:1, v/v) recorded the highest significant percentage values of plantlets survival (67 %) and number of leaves/plantlet (3.1) as well as the highest values of leaf area (15.2 cm2) after 3 consecutive months in acclimatization. Produced saplings from acclimatization showed perfect match and high similarity growth in the greenhouse.

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

INTRODUCTION

Date Palm (*Phoenix dactylifera* L.) is dioecious perennial species (2n=36) of the *Arecaceae* and is one of important *liliopsidaous* fruit crop in Arab countries and Middle East. The family *Arecaceae* comprises about 200 genera and 2500 species distributed through tropical and subtropical regions around the world (FAO statistics 2016). Traditional propagation either by seeds or offshoots, propagation by seeds is undesirable due to a prolonged period of juvenility and a great degree of genetic variation. Propagation by offshoots considered as slow method and in short supply (Zaid and De Wet, 2005). Date palm micropropagation was extensively studied via different explants material. Several workers attempted to culture shoot tip (Al-Khayri and Al-Bahrany, 2001; Ibrahim and Hegazy, 2001; Hegazy,2014b), leaf primordial (Hegazy *et al.* 2006; Hegazy and Aboshama, 2010) and floral bud (Drira and Benbadis 1985 and Hegazy, 2008 ; 2014c).

Date palm was proved to be capable of high frequency regeneration *in vitro* by embryogenesis and organogenesis. A mixed embryogenesis/organogenesis method could also eliminate the risk of abnormalities development (Ferry et al., 2000). Somatic embryogenesis is defined as a process develops from a non-cell without vascular connection with the original tissue (Arnold *et al*., 2002). Somatic embryos could be formed indirectly through callus (Tisserat, 1984) or directly without any intervening callus phase (Hegazy, 2003, McCubbin and Zaid, 2007, Hegazy and Aboshama, 2010). Phillips and Hubstenberger (1995) reported that repetitive embryogenesis showed the best balance of high propagation rates with relatively few off-types. 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). One of the main technological factors limiting the use of indirect embryogenesis technique is the production of abnormal plants (Ferry, 2011). Transfers of plantlets to greenhouse are depending primarily upon the quality and type of materials produced in the previous stages (Hegazy, 2008; 2014a). Plantlets performance during acclimatization was determined to a large extent by the degree of autotrophy (Kozai, 1993).

The aim of this work was to find the most suitable medium composition for propagate date palm cv. Gondella (dray cultivar > 20% moisture content in their fruits) *in vitro* through direct embryogenesis bathway to overcome the short supply of true to type offshoots, enhance distribution and diffusion of high quality cultivar in Aswan region and south Egypt.

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Department of the Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University, during the period 2012- 2015. Leaf primordial explant were obtained from 3 years- old offshoots of date palm cv. Gondella have high quality fruits grown at Abo El. Reech area, Aswan governorate, Egypt.

Medium composition:

The MS basal medium (Murashige and Skooge, 1962) was used occasionally and in other *in vitro* stages was modified (MMS) with ascorbic acid (75 mg/L), citric acid (75 mg/L), Polyvinylpyrrolidone (1.5 g/L), NaH_2PO_4. $2H_2O$ (170 mg/L), putrescine (50 mg/L), glutathione (10 mg/l), asparagen (50 mg/L), activated charcoal (1.5 g/L) and sucrose (40 g/L) solidified with gelrite (2.0 g/L) was used. The pH of the media was adjusted to 5.6 with 0.1 M KOH or 0.1 M HCl prior to gelling agent addition. Media were dispensed either in a glass tubes (25 x 2.5 cm; Borosil) capped with Bellco plastic caps containing 15 ml or into jars (150 ml) at the rate of 40 ml/jar and autoclaved at 121°C and 1.2 Kg/cm² for 20 min.

Explant Preparation:

The outer leaves and fiber sheath were removed from offshoots acropetally with a hatchet and sharp knife until reached 3.0 - 4.0 cm in width and 6.0 - 8.0 cm in length. Then, selected plant tissue spraying with etheyl alcohol 70 % and transfer under aseptic conditions area, plant materials were surface sterilized 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 tree times with sterile distilled water followed by immersed in sterilized mercuric chloride solution (0.1%) for 10 min. It was then rinsed with sterile distilled water and soaked in a filter sterilized antioxidant solution (citric acid (150 mg/L) and ascorbic acid (150 mg/L). Finally, selected plant tissue was kept in the refrigerator (8°C) for 2 hours prior to shorten. Additional leaves were removed one by one till reach shoot tip (1.0 cm²) then leaves primordial were isolated acrobetally one by one and cultured.

1- Effects of growth regulators omition in starting stage:

Leaves primordial explants were cultured individually on MS basal medium supplemented with ascorbic acid (75 mg/L), citric acid (75 mg/L), Polyvinylpyrrolidone (1.5 g/L), NaH2PO4.2H2O (170 mg/L), activated charcoal (1.5 g/L), Yeast extracts (1.0 g/L) [Duchefa Biochemicals Brand (Y1333) and sucrose (40 g/L) solidified with gelrite (2.0 g/L). Nine test tubes replicates were used for etch treatment. Cultures were incubated in total darkness in a growth room at 25±1°C. After 1, 2 and 3 weeks data of Survival %, degree of oxidative browning, explant morphology response were recorded.

2- Effect of growth regulators on direct embryos formation:

Leaves primordial explants obtained from the previous experiment were cultured individually on modified MS medium (MMS) supplemented with cytokinins [2iP (6-(ŷ,ŷ-dimethylallylamino purine, Kin (6-Furfurylaminopurine) and BA (6-benzylaminopurine) and auxin [NOA (Naphthoxy acetic acid)] and [ABA (Abscisic acid) were used (mg/L) at the following concentrations. 2iP at 0.0, 1.0, 3.0 and 5.0 mg/L in combinations with [Kin (0.5): BA: (0.1) NOA (0.25): ABA (0.05) and solidified with gelrite (2 g/L). Nine test tubes/jars (replicates) were used for etch treatment. Cultures were incubated in total darkness for 2 months at 25±1°C. and re-cultured at the same medium 4 weeks intervals. Then, exposed to a 16-h photoperiod using fluorescent tubes with a light intensity of 1500 Lux for another 2 months and re-cultured 4 weeks intervals. After incubation period, data of fresh weight (g), growth value and numbers of formed embryos were recorded.

3- Effect of Ca-pantothenate concentrations on direct embryos growth

Produced embryos obtained from the previous treatment were cultured on MMS medium in addition to Ca-pantothenate at the concentration of 0, 10, 20 and 30 (mg/L). Nine jars (replicates) were used for each treatment. Incubation was carried out under the same embryos formation conditions in light and temperature. After 4 weeks, number of embryos, multiplication rate, number of leaves and leaf area (cm2) were recorded.

4- Effect of spermidine on root formation:

Healthy shoots were individually cultured on basal MS medium supplemented with sucrose (30 g/L) in addition to IBA (indole-3-butyric acid) at the concentration of 0.3 (mg/L) and NAA (Naphthalene acetic acid) at the concentration of 0.3 (mg/L) in combinations with spermidine at the concentration 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 8 weeks, number of leaves, leaf area (cm^2), number of roots and root length (cm) were recorded.

5- **Effect of mixture types on plantlet survival in acclimatization stage:**

Produced plantlets from rooting medium were transferred from test tubes and rinsed under tap water. Plantlets immersed in tap water for 2 h. The root system was immersed in fungicide (Moon cut) solution (0.2 %, w/v) for 5 min. Then, planted in plastic pots (5 ×18 cm) filled with mixture as follows: sugar cane mud alone, sugar cane mud + sand (1:1, v/v), sugar cane mud + peat moss (1:1, v/v), sugar cane mud + rice shell (1:1, v/v), sugar cane mud + Perlite (1:1, v/v), sugar cane mud + bark chips (1:1, v/v) and sugar cane mud + Coconut shell (1:1, v/v). Saplings 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 (75-85 %) 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 (Stimifol, 1.0 g/L) at a ratio of 19:19:19 After 3 months survival %, number of leaves and leaf area (cm^2) 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), high browning (3).

Explant morphology response: It was determined according to the rate of scalling by Pottino (1981), which included, no response (1), positive response (1.5), high response (2.5)

Growth value: Embryos growth value were estimated according to the equation of **Ziv (1992).** $GV = \frac{Fw_F - Fw_i}{Fw_i}$ Where's

GV = Growth value. Fw_F = Final fresh wt. Fw_i = Initial fresh wt.

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

RESULTS AND DISCUSSION

1- Effects of growth regulators omition in starting stage:

As a result of reviewing a large numbers of date palm micropropagation studies, which demonstrated the in vitro pathways and morphogenesis obtained from different date palm explants, it is predicted, this is a pioneer report in using medium free- hormone for explant tissue recovery in the estaplishment stage to initiate direct somatic embros.

Data presented in Table (1) and Fig.1 (a &b) obtained that Date palm dry cv. Gondella offshoots were shortend and immersied in antioxidant. Leaf primordial explant grown after 2 weeks from cuturing in vitro in MS basal medium (free-hormone) treatment were atcheved explant tissue recovery and greatly survival as well as recorded the lowest degree of oxidative browning (1.1) as compared with 3 weeks cultures (2.89) and possitve explant morphology values (1.78) during *in vitro* establishment stage.

In this regard, Virginia and Patricia (1988) have found that less phenolic exudate was released from explants after 24 h in media containing high level of ascorbate and citrate. Pierik (1989) found that browning often making growth and development impossible. Zaid (1984) and Ibrhim and Hegazy (1999) reported that browning of date palm explant tissues could be prevented by pre-soaking of explants in an antioxidant solution. In addition, Hegazy (2003) reported that a date palm leaf primordial explant tissue is accompanied by secretion of the substances into the medium, which accumulates in the culture. These substances, such as phenolic compounds, may have profound physiological disorders on the cultured tissues. Lowering concentration of both citric and ascorbic acids from 150 and 100 mg/l, respectively to the level of 75 mg/l for each significantly decreased degree of date palm explant oxidative browning. In this concern, Ziv (1991) stated that in vitro explant culturing necessitates a continuous supply of growth regulators to the culture medium. The most commonly used growth regulators to the culture medium are auxins and cytokinins supplied either singly or in combination at diverse ratios, depending on the species and the type of explant.

2- Effect of growth regulators on direct embryos formation:

Results in Table (2) and Fig.1(c) showed that shoot tips recultured 3 times on modified MS medium (MMS) supplemented with 2iP (5.0 mg/L) in combination with kin (0.5 mg/L) , BA (0.1 mg/L), NOA (0.25 mg/L) and ABA (0.05) were recorded the highest significant values of fresh wt. (3.63 g) and growth values (3115). This was accompanied by the highest number of direct embryos formatin (0.33) as compared with the control or the other studied treatments. In this regard, Beauchesne *et al*., (1986) found that, at the bottom of the young leaves some very little axillary buds are often visible. Auxins at low concentration, enhanced date palm bud growth in vitro after four to six months, gave some signs of budding which is indicates giving true- to-type plantlets. Very early (Tisserat, 1979) reported that 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 Also, Mater (1986) reported that high auxin levels in the medium favored callus growth and low auxin levels favored normal vegetative growth of date palm shoot tip. Hegazy, 2003) reported that the critical examined factors which could mainly affect the production of somatic embryo from leaf primordial explants, are the type, ratio as well as concentration of both auxin and cytokinin Jiaqiang *et al*. (2003) reported that cytokinin plays a critical role in plant growth and development by stimulating cell division and cell differentiation. Tokuji and Kuriyama (2003) reported that cytokinin regulates the early stage of auxin-induced somatic embryogenesis in carrots. Ziv (1991) stated that in vitro explant culturing necessitates a continuous supply of growth regulators to the culture medium i.e. auxins and cytokinins supplied either singly or in combination at diverse ratios, depending on the species and the type of explant. Researchers founds that Somatic embryos could be formed indirectly through callus (Tisserat, 1984) or directly without any intervening callus phase (Hegazy, 2003 and McCubbin and Zaid, 2007).

Direct somatic embryos of date palm (Phoenix dactylifera L.), " Medjool " spontaneously developed on the individually proliferated axillary buds (Hegazy and Aboshama 2010). 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. Zaid and Tisserat (1983a) reported that, better date plam shoot tip development occurred in vitro on 10 and 100 mg/L NAA.

3- Effect of Ca-pantothenate on embryos multiplication rate and growth characters:

Results obtained in Table (3) and Fig. 1 (d) demonstrated that direct embryos obtained from previous treatment were cultured on MMS medium in addition to Ca-pantothenate at the concentration of 20 (mg/L) scored the highest significant values of multiplication rate (4.24) and the highest No. of leaves (4.00) and leaf area (6.22 cm^2) as compared with the control (2.54), (2.88) and (5.56 cm^2) respectively or the other studied concentration treatments. So far raised Ca-pantothenate concentration from 20 to 30 mg/L reflected significant reduced in multiplication rate from (4.24) to (3.44) and slightly reduced No. of leaves from (4.00) to (3.89) and leaf area from (6.22 cm^2) to (6.00 cm^2). This results strongly confirmed that the vitamin clear effect depends on concentrations. This agreed with the early mentioned vitamins cocentrations effect

In early work, the requirements of tissue cultures for trace amounts of certain organic substances were satisfied by “undefined” supplements such as fruit juices, coconut milk, yeast or malt extracts and hydrolysed casein. These supplements can contribute vitamins.

Pantothenic acid plays an important role in the growth of certain tissues and stimulated tissue proliferation (Huang and Murashige, 1977). Vitamins in culture media should be further studied in order to justify their addition. In the last few decades, little interest has been observed in studying certain vitamins, such as biotin and pantothenic acid. Plant species and cultivars require different amount of vitamins, while other do need any at all (Abrahamian and Kantharajah, 2011). Ca-pantothenate at the concentration of (10 mg L) was superior in the modified MS medium in date palm. Recently, Hegazy and Aboshama (2010), Hegazy (2014b&c) on date palm in vitro cultures reported that using Ca-pantothenate (10 mg/L) was superior in repitative embryos medium, axillary bud proliferation medium and adventitious buds proliferation medium.

4- Effect of spermidine on root formation:

Data represented in Table (4) and Fig.1(e) indicated that, induvedualy healthy shoots cultured on basal MS medium in addition to IBA (indole-3-butyric acid) at the concentration of 0.3 (mg/L) and NAA (Naphthalene acetic acid) at the concentration of 0.3 (mg/L) in combinations with spermidine at the concentration 100 (mg/L) were recorded the highest No. of leaves (4.11) and leaf area (15.56 cm) as well as te highest significant values of No. of roots (3.22) and root length (4.89 cm) as compared with the control or the other studied concentrations 0,50 and 150 mg/L. In micropropagation of various plants NAA is typically added to a media containing nutrients essential to the plants survival. It is added to help induce root formation in various plant types. NAA has been shown to greatly increase cellulose fiber formation in plants when paired with another phytohormone called gibberellic acid. IBA known as a major synthetic auxin used commercially for the induction of adventitious roots and naturally occurring compound derived from IAA via a chain elongation reaction. IBA is also converted to IAA. Reasons for the greater potency of IBA consider unclear. Genetic analysis indicated that high and low rooting responses were probably controlled by multiple genes (Srivastava, 2002). In addition, Sane *et al.* (2006) reported on date palm 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. 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 in most 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.

5- Effect of mixture types on plantlet survival in acclimatization stage:

Data presented in Table (5) and Fig.1(f) indicated that the healthy plantlets produced from rooting stage and submerged to acclimatization stage for 3 months on sugar cane mud + Perlite (1:1, v/v) were recorded the highest No. of leaves (3.10), leaf area (15.20 cm^2), No. of roots (5.33), root length (16.67 cm) and survival (66.67 %) as compared with the control or the other studied concentration treatments. Sugar cane mud is composing organic homogeneous matter contained several essential elements which in turn improved the soil physical condition and consider appropriate compromise medium to support vitroplants growth. In this concern, Hegazy, (2014a) announced that the process between rooting and acclimatization is known *in vitro* hardening. It is a very important step to complete propagation process and raised survival percentage. In addition, (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. That could be lead to the highly mineral availability offered from soil to plants and healthy growth of plants. Marschner, (1995) found that the uptake systems for nutrients are regulated by demand from the shoot. This feedback regulation also extends to enzymes involved in nutrient assimilation and the mobilization of nutrients from the rhizosphere (Raghothama, 2000).

On the other hand, It could be noticed that control treatment (sugar cane mud) alone was destroid all saplings samples and had no survival % at all. This maybe due to poor aeration space as a result to very high moustery content around saplings treatment. In this regard, Hegazy, (2014a) reported that mixture represents the main source of water and nutrients for growing roots. Therefore, it must retain enough moisture, has sufficient porous so that excess water drains away, permitting adequate aeration to the roots and finally remains the nutrients in available form for plants to uptake. Thus, it appears that growing mixture consisted of sand + coarse sawdust (2:1, v/v) could spare the aforementioned requirements, since sand could provide sufficient porous and permit adequate aeration while coarse sawdust may release represent permanent source of available nutrients for the growing plants, which was reflected on the growth characters in parallel with the chemical analysis recorded.

In general, results considered very helpful to the researchers and highly supportive to the nurseries at the commercial level. Since introduce effective and cheapest natural raw materials to fulfillment of the huge demand of insufficient big quantities required. In addition, it has national environment great beneficial effect through implement sugar cane mud good practical recycling and environmental bioremediation and finally reduces production costs.

LITERATURE CITED

Abrahamian. P and Kantharajah. A. 2011. Effect of Vitamins on *In Vitro* Organogenesis of Plant. American Journal of Plant Sciences, 2: 669-674.

Al-Khayri, J. M. and Al-Bahrany, A. M. 2001. Silver nitrate and 2-isopentyladenine promote somatic embryogenesis in date palm (Phoenix dactylifera L.). Sci. Hort., 89: 4, 291-298.

AL-Mayahi, A. M. 2014. Effect of copper sulphate and cobalt chloride on growth of the in vitro culture tissues for date palm (Phoenix dactylifera L.) cv. Ashgar. American Journal of Agricultural and Biological Sciences 9 (1): 6-18, 2014

A.Navalón, R. Blanc, J.L. Vilchez Determination of 1-naphthylacetic acid in commercial formulations and natural waters by solid-phase spectrofluorimetry Mikcrochim. Acta, 126 (1997), pp. 33–38

Arnold, S.; Sabala, I.; Bozhkov, P.; Dyachok, J. and Filonova, L. 2002. Developmental pathways of somatic embryogenesis. Plant Cell Tiss. Org. Cult. 69: 233-249.

Beauchesne, G.; Zaid, A. and Rhiss, A. 1986. Meristematic potentials of bottom of young leaves to rapidly propagate date palm. Proc. Second Sym. Date Palm, Al-Hassa, Saudia Arabia p. 87- 93.

Drira, N. and Benbadis, A. 1985. Vegetative multiplication of date palm (*Phoenix dactylifera* L.) by reversion of in vitro cultured female flower budy. J. Plant Physiol. 119: 227-253.

Ferry, M. 2011. Potential of Date Palm Micropropagation for Improving Small Farming Systems . In Date Palm Biotechnology (Jain, S. M. *et al.* (eds.), Springer Dordrecht Heidelberg London New York. 15- 28.

F. A. O. 2016. Food and Agriculture organization. Agro-Statistics Database.

Handa, A. and Mattoo, A 2010. Differential and functional interactions emphasize the multiple roles of polyamines in plants. Plant Physiology and Biochemistry 48 (2010) 540e546

Hegazy, A. E. 2003. Some Physiological Studies on Date Palm Micropropagation Through Direct Somatic Embryogenesis. Ph. D. Thesis. Plant Physiol. Dep. Fac. of Agric. Cairo Univ. Egypt.

Hegazy, A. E. 2008. Micropropagation of Egyptian date palm c.v Selmy through floral buds culture. J. Agri. Sci. Mansoura Univ. 33 (4): 2803-2815**.**

Hegazy, A. E. 2014a. In vitro hardening affect growth in the nursery of date palm cv. Medjool vitroplants. Arab. J. Biotech. 27 (2): (Accepted).

Hegazy, A. E. 2014b. Promisin g protocol for in vitro direct organogenesis of date palm cv. Ekhlass. Fifth International Date Palm Conference , Abu Dhabi - UAE; 16 – 18 March.

Hegazy, A. E 2014c. In vitro propagation of selected aged date palm male clone via direct adventitious buds proliferation. Egypt. J. Biotech. Oct., Vol. 47: 71-86.

Hegazy, A. E. and Aboshama, H. M. (2010). An efficient novel pathway discovered in date palm micropropagation. Acta Hort. (ISHS) 882:167-176.

Hegazy, A. E.; Ahmed, O. K.; Abul-Soad , A. A. and Nasr, M. I. (2006 a). Growing Behaviors of ex vitro Date Palm Plants after Acclimatization. The 2nd International Conference of Genetic Engineering& Its Applications, Sharm El-Sheik City, South Sinai, Egypt; 14-17 November. p 15.

Huang, L. and Murashige, T. (1977) Plant Tissue Culture Media: Major Constituents, Their Preparation and Some Applications. TCA Manual/Tissue Culture Association, 3, 539-548.

Ibrahim, I. A and Hegazy , A. E. (1999). Micropropagation of date palm (Phoenix dactylifera L.cv. Samany) via direct organogenesis. The First International Conference in Egypt on Plant Tissue Culture and its Application, pp. 201-207.

Ibrahim, I. A. and Hegazy, A. E. 2001. In vitro cultivation of date palm. 1- Micropropagation of adult and juvenile date palm. Mid-Atlantic plant molecular Biology Society, Eighteenth Annual Meeting, Beltisville-MD, USA, 2-3 Aug. p. 37.

Jiaqiang Sun, Qi-Wen Niu, Petr Tarkowski, Binglian Zheng, Danuse Tarkowska, Göran Sandberg, Nam-Hai Chua, and Jianru Zuo (2003). The Arabidopsis AtIPT8/PGA22 Gene Encodes an Isopentenyl Transferase That Is Involved in De Novo Cytokinin Biosynthesis. Plant Physiol., 131: pp. 167-176.

Kozai, T. 1993. Micropropagation under photoautotrophic conditions . In micropropagation. Technology and Application (Debergh P.C. and Zimmerman, R.H. eds). Kluwer Academic , Netherland, pp. 477-469.

Marschner, H. 1995. Mineral nutrition of higher plants. London: Academic Press.

Mater, A. 1986. In vitro regeneration of (*Phoenix dactylifera* L) Date Palm J. 4 (2): 137-152.

McCubbin, M. J. and Zaid, A. 2007. Would a combinations of organogenesis and embryogenesis techniques in date palm micropropagation be the answer? Acta Hort. 736:255-259

Murashige, T. and Skoog, F. A. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15: 433-479.

Phillips, G. C. and Hubstenberger, J. F. 1995. Micropropagation by proliferation of axillary buds. P. 45-54. In: O. L. Gamborg, and G. C. Phillips (eds.), Plant Cell, Tissue and Organ Culture, Fundamental Methods, Springer-Verlag Berlin.

Picoli, E. A.; Otoni, W. C.; Figueira, M. L.; Carolino, S. M.; Almeida, R. S.; Silva, E. A.; Carvalho, C. R. and Fontes, E. P. 2001. Hyperhydricity in in vitro eggplant regenerated plants: structural characteristics and involvement of BiP (Binding Protein). Plant Sci. 160: 5, 857-868.

Pierik, R. L. M. 1989. In vitro culture of higher plants. Martinus Nijhoff publishers, Netherlands.

Pottino, B. G. 1981. Methods in Plant Tissue Culture. Dept. of Hort., Agric., Maryland Univ., College Park, Maryland , USA, pp. 8-29.

Raghothama, K. G. 2000. Phosphate transport and signaling. Current Opinion in Plant Biology 3, 182–187.[Medline]

Sane, D.; Aberlenc-Bertossi, F.; Gassama-Dia, Y.; Sagna, M.; Trouslot, M.; Duval, Y. and Borgel, A. 2006. Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of date palm (*Phoenix dactylifera* L.). Annals of Botany 98: 301- 308.

SAS, 1988. Statistical analysis system SAS User's Guide: Statistics SAS Institute Inc., Cary, N. S., ed.

Srivastava, L. M. 2002. Plant Growth and Development: Hormones and Environment. Academic Press. An imprint of Elsevier, San Diego, California, 245-248.

Steel, R. G. and Torrie, J. H. 1980. Principles and Procedures of Statistics, a Biomerical Approach. Mc Grow- Hill Book Company, New York, 469-517.

Tisserat, B. 1979. Propagation of date palm (*Phoenix dactylifera* L.) in vitro. J. Exp. Bot. 30 (119): 1275-1283.

Tisserat, B. 1984. Propagation of date palm by shoot tip cultures. Hort. Sci. 19:230-231.

Tokuji, Y. and Kuriyama, K. (2003). Involvement of gibberellin and cytokinin in the formation of embryogenic cell clumps in carrot (Daucus carota). J. Plant Physiol., 160 (2): 133-141.

Virginia, H. and Patricia, M. H. 1988. Factor effecting the release phenolic compounds explants of (Polargonium hortorum), Bailey sprinter scarlet. J. Horit., 63(4): 651-657.

Zaid, A. 1984. In vitro browning of tissues and media with special emphasis to date palm cultures. A review. Date Palm J., 3 (1): 269-275.

Zaid, A. and De Wet, P. 2005. Date palm propagation. Chapter 5 In: Zaid, A. Date Palm Cultivation. FAO plant production and protection. Vol.156.

Zaid, A. and Tisserat, B. 1983a. In vitro shoot tip differentiation in (*Pheonix dactylifera*L.). Date Palm J. 2 (2): 163-182.

Ziv, M.1991. Virtification morphological and disardors of in vitro plants. P. 45-69. In: P.C. Deberegn and R.H. Zimmerman (eds.), Micropropagation technology and application, Kluwer Academic publishers Dordrecht. Boston, London.

Ziv, M. 1992. The use of growth retardants for the regulation and acclimatization of in vitro plants. P. 809-817. In: C.M. Karssen; L.C. Van loon and D. Vreugdenhil, (eds), Progress in Plant Growth Regulators.

Tables

Table (1): Effect of MS basal medium (free-hormone) treatment on explant tissue recovery during *in vitro* establishment stage of date palm cv. Gondella

Incubation Period (Weeks)	Survival %	Degree of oxidative browning (*)	Explant morphology response (**)
One	100	1.00^{b}	1.00^{b}
Two	100	1.10^{b}	1.78^{a}
Three	100	2.89^{a}	2.00^{a}
L.S.D$_{0.05}$	0.00	0.26	0.25

Means within each column followed by the same letter are not significantly (N.S) different at P= 0.05 according to the LSD test. **Rating Scale*** : **1**- No browning. 2- Average browning. 3-High browning
Rating Scale** 1-No Response 1.5- Positive Response 2.5- high response

Table (2): Effect of 2iP concentrations in combinations with Kin, BA, NOA and ABA on direct embryos formation of date palm cv. Gondella sub-cultured *in vitro* for 4 months.

Treatments (mg/L)		Explant growth characters			
		Fresh weight (g)		Growth Value	Number of formed embryos
		Start	Final		
2 iP (0.0)	**Kin (0.5) + BA (0.1) + NOA (0.25) + ABA (0.05)**	0.10^{a}	0.94^{d}	784^{d}	0.00^{a}
(1.0)		0.11^{a}	1.41_{c}	1185^{c}	0.00^{a}
(3.0)		0.10^{a}	1.88^{b}	1768^{b}	0.00^{a}
(5.0)		0.11^{a}	3.63^{a}	3115^{a}	0.33^{a}
L.S.D$_{0.05}$		0.01	0.23	255	0.34

Means within each column followed by the same letter are not significantly (N.S) different at P= 0.05 according to the LSD test.

Table (3): Effect of Ca-pantothenate concentrations on repitative embryos of date palm embryos cv. Gondella cultured *in vitro* for 4 weeks.

Treatments (mg/L)	Number of embryos		Multiplication Rate	No. Of Leaves	Leaf area (cm^2)
	Start	final			
Ca-pantothenate 0.0	9.22^{a}	23.44^{d}	2.54^{d}	2.88^{b}	5.56^{a}
10.0	9.11^{a}	28.67^{c}	3.15^{c}	3.11^{b}	5.89^{a}
20.0	9.44^{a}	40.00^{a}	4.24^{a}	4.00^{a}	6.22^{a}
30.0	9.56^{a}	32.89^{b}	3.44^{b}	3.89^{a}	6.00^{a}
L.S.D$_{0.05}$	0.77	0.88	0.59	0.28	1.30

Means within each column followed by the same letter are not significantly (N.S) different at P= 0.05 according to the LSD test.

Table (4): Effect of spermidine in combination with auxins on root growth characters of date palm shoots cv. Gondella cultured *in vitro* for 8 weeks

Treatments (mg/L)		No. Of Leaves	Leaf area (cm^2)	No. Of Roots	Root length (cm)
IBA (0.3 mg/L) + NAA (0.3 mg/L)	**Spermidine** 0.0	3.22^{b}	13.67^{b}	1.89^{c}	1.89^{d}
	50.0	3.56^{ab}	14.00^{ab}	2.11^{c}	3.11^{c}
	100.0	4.11^{a}	15.56^{a}	3.22^{a}	4.89^{a}
	150.0	4.00^{a}	15.22^{ab}	2.67^{b}	4.33^{b}
L.S.D$_{0.05}$		0.55	1.31	0.46	0.44

Means within each column followed by the same letter are not significantly (N.S) different at P= 0.05 according to the LSD test

Table (5): Effect of mixture types on survival percentage and growth characters of date palm cv. Gondella plantlets after 3 months in acclimatization.

Mixture treatments		No of leaves	Leaf area (cm^2)	No. of roots	Root length (cm)	Survival %
Sugar cane mud		**0.00^{a}**	**0.00^{a}**	**0.00^{b}**	**0.00^{a}**	**0.00^{b}**
	+ Sand (1:1, v/v)	**2.36^{a}**	**10.73^{a}**	**3.67^{ab}**	**14.33^{a}**	**33.33^{ab}**
	+ Peatmoss (1:1, v/v)	**2.63^{a}**	**11.23^{a}**	**2.00^{ab}**	**13.40^{a}**	**20.00^{b}**
	+ Rice shell (1:1, v/v)	**2.50^{a}**	**11.83^{a}**	**4.47^{ab}**	**13.65^{a}**	**26.67^{ab}**
	+ Perlite (1:1, v/v)	**3.10^{a}**	**15.20^{a}**	**5.33^{a}**	**16.67^{a}**	**66.67^{a}**
	+ Bark chips (1:1, v/v)	**2.77^{a}**	**13.07^{a}**	**3.80^{ab}**	**15.60^{a}**	**40.00^{ab}**
	+ Coconut shell (1:1, v/v)	**2.47^{a}**	**12.33^{a}**	**2.20^{ab}**	**13.80^{a}**	**20.00^{b}**
L. S. D$_{0.05}$		**2.78**	**10.62**	**3.51**	**15.29**	**31.06**

Means within each column followed by the same letter are not significantly (N.S) different at P= 0.05 according to the LSD

Figures

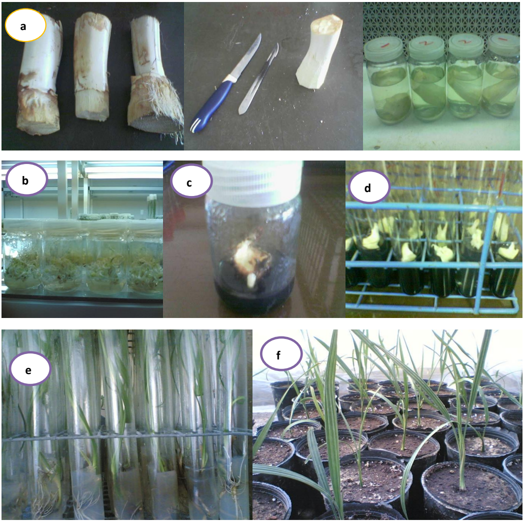

Fig. 1. Date palm dry cv. Gondella micropropagation
a- Shortend offshoots and immersion in antioxidant. **b**- Leaf primordial explant grown after 2 weeks from cuturing . **c-** Direct embryo formation in vitro on explant after 4 months under dark/light conditions on modified MMS. d- High Repitative embryos grown in vitro on modified MMS medium supplimente with Ca-pantothenate (20 mg/L) for 4 weeks. **e-** Well developed plantlets in rooting medium contained *IBA (0.3 mg/L) , NAA (0.3 mg/L), spermidine (100 mg/L* and obtained healthey root system. **f**- Date palm vitroplants in acclimatixzation stage on mixture containg Sugar cane mud + Perlite (1:1, v/v).

Micropropagation of cv. Dhakki a high value date palm cultivar of Pakistan using offshoot and inflorescence explants

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Abstract

Immature inflorescences and shoot tip explants were used to propagate cv. Dhakki of date palm, a high value cultivar with limited distribution in Pakistan. The inflorescence explants found with less browning and mortality issues with higher callus (80.22%) and embryo formation (69.91%), and average shoot formation (81.22%) than shoot tip explants. Whereas, there was no significant differences recorded in terms of average root formation and plantlet formation, and survival percentages in both type of explants. The strong rooted plantlets were acclimatized successfully in green house and then transferred to field conditions. The primary evaluation in terms of morphological characterization have not showed any somaclonal variation in tissue culture derived plantlets of cv. Dhakki.

Keywords: Dhakki, inflorescence, micropropagation, PGRs, *Phoenix dactylifera* L.

INTRODUCTION

With production of 8.4 million tons and 1.3 million hectares area under cultivation (FAO, 2016), date palm is one of the important horticultural commodity of the world. The major dates producing areas lies under the warmer regions worldwide especially Gulf, Northern Africa and West of Asia (Pakistan) while some dispersed cultivation is also found almost in every continent (except Antarctica). The advancement of tissue culture technology enables the cultivation of date palm in some non-dates producing countries as well such as Afghanistan, Kenya, Namibia, South Africa, Bangladesh, Thailand etc. (Abul-Soad et al., 2017).

Pakistan is blessed with rich varietal diversity of date palm and has historical background of dates cultivation dated back to Indus Civilization (2900 BC) (Marshal, 1931; Jatoi et al., 2009; Jatoi, 2013). Dates found in all four provinces of Pakistan, and each province has its own elite exporting cultivars such as cvs. Aseel and Karblain (Sindh), cvs. Begum Jangi and Muzawati (Balochistan) and cv. Dhakki (Khyber Pakhtunkhwa). The cv. Dhakki is a recent addition in the major dates exporting varieties from Pakistan mainly cultivated in Dera Ismail Khan of Khyber Pakhtunkhwa province and got popularity due to its attractive size (4-5 cm in length and 2-3 cm in thickness) and excellent sensory properties. Since then its cultivation started in other provinces of Pakistan but the availability of enough offshoots of cv. Dhakki is the major hindrance towards its rapid commercial propagation throughout the country. Its propagation using tissue culture technology is the possible solution to overcome the shortage and risk of transmitting pest and pathogens which is one of major issue often comes with traditional propagation of date palm.

The history of date palm tissue culture is extended over last four decades but limited to basic research on small scale (Schroeder, 1970; Reuveni and Lilien-Kipnis, 1974; Tisserat, 1979), the studies on commercial or large-scale production of date palm plantlets started later (Zaid et al., 2007; Abul-Soad and Mahdi, 2010; Jatoi et al., 2015). The offshoot derived explants are more popular type of explants used for propagation of date palm worldwide. The tissue culture protocol using offshoots often hampered by heavy browning, bacterial contamination and high mortality rate in addition to the limitation of offshoots in case if propagation is desired of the single seeded tree having no offshoots. The potential of inflorescence explants has been tested by several researchers to establish successful protocol of date palm propagation (Bhaskaran and Smith, 1992; Fki et al., 2003; Zaid et al., 2007; Abul- Soad and Mahdi, 2010; Jatoi et al., 2015).

Tissue culture studies of date palm in Pakistan started three decades ago but was limited to somatic embryogenesis as basic research resulting in fewer number of plantlets reached at acclimatization and transfer to field conditions (Rashid and Quraishi, 1994; Khan and Bibi, 2012). Date Palm Research Institute (DPRI), Shah Abdul Latif University, Khairpur is the pioneer in establishing successful micropropagation protocols of number of elite cultivars of date palm in Pakistan (Abul-Soad and Mahdi, 2010; Abul-Soad, 2011; Jatoi et al., 2015). The aim of current study was the in vitro production of this high value and high demand date palm cultivar in the region utilizing both offshoot and inflorescence derived explants.

MATERIAL AND METHODS

The experiments were conducted at Plant Tissue Culture Laboratory of Date Palm Research Institute (DPRI), Shah Abdul Latif University (SALU), Khairpur, Pakistan during 2012 - 2016.

Plant Material

The offshoots were acquired from the adult trees grown at research nursery of Date Palm Research Institute (DPRI), SALU, Khairpur, Pakistan while, the inflorescences were brought from Dera Ismail Khan, Khyber Pakhtunkhwa province of Pakistan.

Explant Preparation and Sterilization Process:

The immature inflorescences were excised from the mother trees of cv. Dhakki in early spring and kept in clean plastic cover and handled carefully from an open field to the laboratory conditions. The intact spathes were dipped into fungicide solution (2 $g.L^{-1}$ Topsin M 70) for 30 seconds only, without shaking followed by washing under current tap water for 30-60 seconds. The spathes were simply sterilized using spirit heat up under aseptic conditions (Fig. 1A), then the outer protective sheath or cover was removed and the spikelets were cut from their bases and cultured on nutrient medium.

Whereas, the offshoots (2-3 years old) were carefully removed from the mother trees and then reduced in size by removing the fibrous sheath and outer leaves acropetally and dipped into a fungicide solution of Thiovet-jet (2 $g.L^{-1}$ for 5 min). Then, the peeled offshoots brought under aseptic conditions and further reduced and surface sterilized with 50% sodium hypochlorite ($NaOCl_2$) solution with few drops of Tween-20 for 15 min and then with 3 $g.L^{-1}$ mercuric chloride ($HgCl_2$) solution for 5 min and washed three times with sterilized distilled water. After sterilization, the outer soft leaves of apical dome were removed in order to get the terminal portion of shoot apex (1-2 cm) (Fig. 1B) and cultured into the nutrient medium.

Nutrient Medium Protocol and Cultural Conditions

The nutrient medium was prepared as mentioned in Table 1 for each tissue culture stage and dispensed into different vessels as per the tissue culture stage and cultures requirement. All cultures were kept under complete dark conditions at 27±2 °C during initiation stage and were shifted to light conditions as per the culture conditions and requirements. The ideal plantlets with strong root and shoot system were shifted to MS medium devoid of PGRs and agar prior to acclimatization process. Afterward, they were transplanted into plastic bags with peat moss and river sand soil medium for acclimatization process in the glasshouse as per protocol reported by Abul-Soad (2011) and then shifted to field conditions at tissue culture date palm nursery of Shah Abdul Latif University, Khairpur, Pakistan.

Statistical Analysis

A set of measurements like explant mortality rate, average callus and embryo formation, average shoot and root development and ideal plantlet formation were taken after each sub-culture. The sub-culturing was performed after every 4 weeks. Browning, swelling and callus formation is expressed as scores and presented as +, ++, +++, - represent poor, moderate, high and no response, respectively. The survival percentage was recorded after 1 month of transplantation in glasshouse. The data is reported as means ± standard deviation and the significance of differences among means were obtained using LSD test at $p<0.05$ using "Statistix 8.1" software.

RESULTS AND DISCUSSION

The data presented in Table 2 showing significant differences in terms of initiation response of inflorescence and shoot tip explants of cv. Dhakki of date palm. The Inflorescence explants recorded with less mortality rate as compared to shoot tip explants. Moreover, no browning or minimal browning was noticed in inflorescence explants in comparison to heavy browning recorded in shoot tip explants. Whereas, there was no significant difference in both type of explants regarding the swelling of explants. since, polyphenol oxidase is highly occurred in date palm tissues and attributed to the production of phenolic compounds in the culture media that caused oxidative browning of the explants (El-Hadrami, 1995). Hence, the careful handling and proper sterilization protocol are recommended to avoid such problems (Abul-Soad, 2011).

The inflorescence explants formed significantly highest percentages of callus (80.22%) and embryo (69.91%) (Fig. 1C) as compared to shoot tip explants (77.46% and 63.62% callus and embryo formation) in cv. Dhakki of date palm (Table 3). Upon completion of initiation stage, the induction of callus and formation of embryos or indirect and direct somatic embryogenesis mainly depends on the composition of nutrient medium supplemented different plant growth regulators (PGRs) and additives (Jatoi, 2013). Like, Bhaskaran and Smith (1992) induced callus production using shoot tip explants of date palm on MS medium containing 100 mg.L^{-1} 2,4-D + 1 mg.L^{-1} 2iP + 3% sucrose and 3 mg.L^{-1} activated charcoal. Likewise, Tisserat (1979) achieved successful callus induction in shoot tip and lateral bud date palm explants on a medium supplemented with 3 mg.L^{-1} 2iP, 0.1-100 mg.L^{-1} NAA or 2, 4-D and 3 g.L^{-1} AC. Whereas, El-Shiaty (2004) found that adding 50 mg.L^{-1} glutamine or 5 mg.L^{-1} biotin in MS medium induced callus while the higher concentrations resulted in induction of somatic embryos and shoot formation. As per findings of Bader et al. (2007) the formation of callus on floral explants of two date palm cultivars occurred after 12 weeks of incubation under dark conditions while the adventitious bud formed on callus after another 12 weeks of incubation.

Whereas, the data regarding average shoot, root and plantlet formation, and survival percentage of inflorescence and shoot tip explants of tissue culture derived plants of cv. Dhakki of date palm is presented in Table 4. The inflorescence explants appeared with significantly highest percentages of shoot formation (81.22%) than shoot tip explants (78.00%) (Fig. 1D & 1E) but both proliferated well on multiplication medium under light conditions (Fig. 2A). Whereas, there was no significant differences recorded in both explants regarding the root formation and average plantlet formation. However, inflorescence explants produced highest average rooting

(83.14%) and plantlet formation (81.64%). The plantlets were shifted to rooting and elongation medium first with activated charcoal and then without activated charcoal that resulted with strong root and shoot system with secondary and tertiary adventitious root formation (Fig. 2B) and in accordance with the findings of Abul-Soad and Jatoi (2014). Similarly, there was no significant differences recorded among inflorescence and shoot tip derived plants of cv. Dhakki in terms of survival percentages during acclimatization. However, the plantlets derived from both type of explants resulted with more than 90% of survival percentage in glass house conditions (Fig. 2C). According to Zaid et al. (2006) in order to obtain highest multiplication rate, the nutrient medium should contain lower levels of PGRs. Similar type of findings obtained by Al-Khateeb (2006) in micropropagation of date palm (cv. Sukary) where lower concentration of PGRs resulted with higher rates of shoots while higher concentrations shown abnormal development in date palm plantlets. Likewise, Al-Kharyi and Al-Maarri (1997) achieved highest multiplication rate in date palm on MS medium comprised of lower concentration of PGRs in addition of 1.5 $g.L^{-1}$ AC and 6 $g.L^{-1}$ agar. The elongation and rooting phase is needed in order to produce ideal plantlets for highest survival percentage during acclimatization process (Tisserat, 1983; Abul-Soad et al., 1999; Jatoi, 2013; Abul-Soad and Jatoi, 2014; Jatoi et al., 2015). The re-culturing (approx. 8-16 weeks) of individual shoots of date palm on MS medium containing 0.1 $mg.L^{-1}$ NAA may resulted with vigorous adventitious root formation (Tisserat 1984). Whereas, Gabr and Abd-Alla (2010) and Abul-Soad and Jatoi (2014) reported that using polyethylene glycol (PEG) in MS or modified MS medium may produce ideal healthy adventitious rooting formation in date palm plantlets prior to shift them in glasshouse conditions. As per findings of Abul-Soad and Jatoi (2014) that ideal plantlets having adventitious roots with two to three leaves during *in vitro* conditions were proven best to get highest survival percentages of date palm plants during acclimatization process that is in accordance with the results obtained in current study. According to Mazri and Meziani (2013) the culturing of date palm plantlets devoid of PGRs prior to acclimatization process resulted with more than 90% survival percentage with healthy appearance acclimatized plants.
In addition, the tissue culture derived plants of cv. Dhakki with at least one compound leaf were shifted to the field conditions (Fig. 2D) and till now there is no any published phenotypic symptoms of somaclonal variation has been recorded while to ensure the genotypic nature the plants are under evaluation.

In conclusion, the *in vitro* production of cv. Dhakki of date palm utilizing inflorescence and shoot tip explants has been achieved under this study. The responses of both type of explants showed not much differences regarding shoot and root formation and as well as for survival percentages during acclimatization. Nevertheless, the Inflorescence explants showed number of advantages over shoot tip explants such as no or less browning and low mortality rate with higher callus and embryo formation that are most crucial steps in establishing a successful protocol of date palm tissue culture.

LITERATURE CITED

Abul-Soad, A.A. 2011. Micropropagation of date palm using inflorescence explants. pp. 91-118. In: S.M. Jain, J.M. Al-Khayri and D.V. Johnson (eds.), Date Palm Biotechnology, Springer International Publishing, Dordrecht.

Abul-Soad, A.A. and Jatoi, M.A. 2014. Factors are affecting *in vitro* rooting of date palm (*Phoenix dactylifera* L.). Pak. J. Agri. Sci. 51:467-474.

Abul-Soad, A.A. and Mahdi, M.S. 2010. Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. J. Gen. Eng. Biotech. 8: 39-44.

Abul-Soad, A.A., Ibrahim, I.A., El-Sherbeny, N.R. and Baker, S.I. 1999. *In vitro* and *ex vitro* optimization for rooting and acclimatization of date palm. Proc. First International Conference in Egypt on Plant Tissue Culture and its Application. Zagazig, Egypt 12-14 September. pp. 227-241.

Abul-Soad, A.A., Jain, S.M. and Jatoi, M.A. 2017. Biodiversity and conservation of date palm. pp. 313–353. In: M. Ahuja and S.M. Jain (eds.), Biodiversity and Conservation of Woody Plants, Springer International Publishing, Dordrecht.

Al-Kharyi, J.M. and Al-Maarri, K.W. 1997. Effect of seasonal variation on the regeneration capacity of date palm. In vitro. 33:22-26.

Al-Khateeb, A.A. 2006. Role of cytokinen and auxin on the multiplication stage of date palm (*Phoenix dactylifera* L.) cv. Sukry. Biotech. 5:349-352.

Bader, S.M., Baum, M.H., Khierallah, S.M. and Choumane, W. 2007. The use of RAPDs technique for the detection of genetic stability of date palm plantlets derived from *in vitro* culture of inflorescence. Proc. first conference on Biology. University of Mosul, Mosul, Iraq 4-5 September. pp. 149-159.

Bhaskaran, S. and Smith, R.H. 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* cv. Barhee. Plant Cell Rep. 12:22-25.

El Hadrami, I., Cheikh, R. and Baaziz, M. 1995. Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. Biol. Plant. 37:205–211.

EL-Shiaty, O.H., El-Sharabasy, S.F. and Abd-El-Kareim, A.H. 2004. Effect of some amino acids and biotin on callus and proliferation of date palm (*Phoenix dactylifera* L.) Sewy cultivar. Arab J. Biotech. 7:265-272.

FAO. 2016. Food and Agriculture Organization of the United Nations (FAO) Statistics.

Fki, L., Masmoudi, R. Drira, N. and Rival, A. 2003. An optimized protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. Plant Cell Rep. 21:517-524.

Gabr, M.F. and Abd-Alla, M.M. 2010. Micropropagation of *Phoenix dactylifera* L. var. Karama. New York Sci J. 3:64-69.

Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.

Jatoi, M.A. 2013. *In vitro* rooting and acclimatization of date palm (*Phoenix dactylifera* L.) plantlets. M.Phil Thesis, Dept. of Botany, Shah Abdul Latif University, Sindh, Khairpur, Sindh, Pakistan.

Jatoi, M.A., Abul-Soad, A.A., Markhand, G.S. and Solangi, N. 2015. Establishment of an efficient protocol for micropropagation of some Pakistani cultivars of date palm (*Phoenix dactylifera* L.) using novel inflorescence explants. Pak J. Bot. 47:1921-1927.

Jatoi, M.A., Markhand, Z. and Solangi, N. 2009. Dates in Sindh: facts and figures. Proc. International Dates Seminar. Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Pakistan 28 July. pp. 59-71.

Khan, S. and Bibi, T. 2012. Direct shoot regeneration system for date palm (*Phoenix dactylifera* L.) cv. Dhakki as a means of micropropagation. Pak. J. Bot. 44:1965-1971.

Marshal, J. 1931. Mohenjo-Daro and the Indus Civilization: being an official account of Archaeological excavations at Mohenjo-Daro carried by the Govt. of India between the years 1922-27. First published at London, 1931. Reprinted in 2004 by AES Publications Pvt. Ltd, New Delhi.

Mazri, M.A and Meziani, R. 2013. An improved method for micropropagation and regeneration of date palm (*Phoenix dactylifera* L.). J. Plant Biochem. Biotechnol. 22:176-184.

Murashige, T. and Skoog, F.A. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physio. Planta. 15:473-479.

Rashid H. and Quraishi, A. 1994. Micropropagation of date palm (*Phoenix dactylifera* L.) cv. Dhakki through tissue culture. Pak. J. Agric. 15:1-7.

Reuveni, O. and Lilien-kipnis, H. 1974. Studies of the *in vitro* culture of date palm (*Phoenix dactylifera* L.) tissue and organs. Volcani Institute Agricultural Research Pamphlet. 145: 1-40.

Schroeder, C.A. 1970. Tissue culture of date shoots and seedlings. Date Grower's Inst. Rept. 49:25-27.

Tisserat, B. 1979. Propagation of date palm (*Phoenix dactylifera* L.). *in vitro*. Journal of Exp. Bot. 30:1275-1283.

Tisserat, B. 1983. Development of new tissue culture technology to aid in the cultivation and crop improvement of date palms. Proc. first symposium on the date palm. Al-Hassa, Saudi Arabia. pp. 126-139.

Tisserat, B. 1984. Date palm. pp. 505-545. In: W.R. Sharp, D.A. Evans, P.V. Ammirato and Y. Yamada (eds). Handbook of Plant Cell Culture, vol.2, Mc Millian, New York.

Zaid, A., Al Kaabi, H.H. and El-Korchi, B. 2007. Large scale *in vitro* propagation of a rare and unique male date palm (*Phoenix dactylifera*) using inflorescences technique. Acta Hort. 736:243-254.

Zaid, A., Al-Kaabi, H.H. and El-Korchi, B. 2006. Impact of lower concentration of growth regulators on the multiplication stage of date palm organogenesis. Proc. Third International Date Palm conference. Abu Dhabi, UAE. pp. 19-21.

Tables

Table 1. Nutrient media composition (in $mg.L^{-1}$) of different stages of tissue culture for offshoots and Inflorescence explants of date palm (cv. Dhakki).

Medium	Salts	Additives	[5]PGRs & AC	
			Inflorescence	Offshoots
Initiation	Macro of B5[1]+ Micro of MS[2]	30000 Suc.[3] + 2200 Agar + 1400 Gel + Vit.[4] of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Adenine sulfate	0.1 2,4-D + 0.1 IAA + 5.0 NAA	10 2,4-D + 3 2ip + 1500 AC
Maturation	Macro of B5+ Micro of MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Adenine sulfate	5.0 2,4-D + 1.0 2iP + 1500.0 AC[6]	10 2,4-D + 3 2ip + 3000 AC
Differentiation	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA + 0.1 Kinetin	0.1 NAA + 0.1 2ip + 100 AC
Multiplication	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA + 0.05 BA	0.1 NAA + 0.5 2ip + 100 AC
Rooting	¼ MS	40000 Suc. + 2400 Agar + 1400 Gel + Vit. of MS + 0.1 Gibberellic acid (GA3) + 40 Adenine sulfate	0.1 NAA + with & without 3000 AC	0.1 NAA + with & without 3000 AC

[1]B5: Gamborg et al. (1968) nutrient medium. [2]MS: Murashige and Skoog Medium (1962). [3]Suc.: Sucrose. [4]Vit.: Vitamins. [5]PGRs: Plant growth regulators. [6]AC: Activated Charcoal.

Table 2. Response of inflorescence and shoot tip explants on mortality, browning and swelling of explants in cv. Dhakki.

Explant type	Mortality	Browning	Swelling
Inflorescence	+	-	++
Shoot tip	++	+++	++

+, ++, +++, - signs represents poor, moderate, high and no response in explants, respectively

Table 3. Influence of different explant type on callus and embryo formation of cv. Dhakki during initial 4 sub-cultures.

Explant type	Average Callus Formation	Average Embryo Formation
Inflorescence	80.22 ± 8.54^a	69.91 ± 7.89^a
Shoot tip	77.46 ± 10.49^b	63.62 ± 10.83^b

Means on the same column with different superscript letters are significantly different at $p<0.05$.
Means ± standard deviation.

Table 4. Influence of different explant type on shoot formation (shoot cluster), root formation, and survival percentages of cv. Dhakki.

Explant type	Average Shoot formation	Average Root Formation	Average Plantlet formation	Survival % Acclimatization)
Inflorescence	81.22 ± 11.24^a	83.14 ± 9.28^a	81.64 ± 12.25^a	92.24 ± 11.14^a
Shoot tip	78.00 ± 9.55^b	82.16 ± 11.25^a	80.46 ± 9.22^a	91.21 ± 10.15^a

Means on the same column with different superscript letters are significantly different at $p<0.05$.
Means ± standard deviation.

Figures

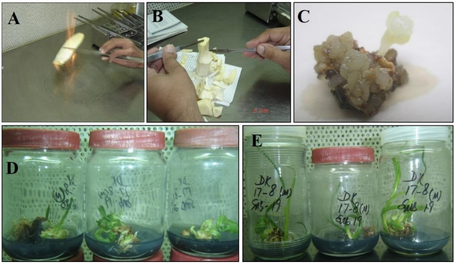

Fig. 1 Different stages of cv. Dhakki tissue culture, (A) Spathe sterilization on spirit heat up, (B) shoot tip explants preparation, (C) callus and embryo formation (D and E) shoot cluster and shoots separation.

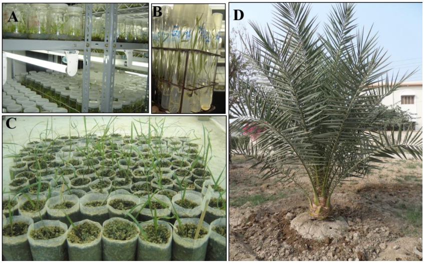

Fig. 2 *In vitro* and *ex vitro* conditions of cv. Dhakki (A) multiplication, (B) Elongation and Rooting, (C) acclimatization process in glasshouse, (D) tissue culture derived plants of cv. Dhakki in field conditions.

Date Palm Micropropagation and its Key Role in the Current Development Strategy of Date Sector in Morocco

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Abstract

Date palm (*Phoenix dactylifera* L.) of the Arecaceae family is a key plantation crop in the arid zones of many countries in the Middle East and North Africa. Out of the total world production of 8.5 million tons of dates, more than 70% are produced by the Arab countries (FAO, 2016).The estimated annual production of dates in Morocco is about 111 000 tons. However, date palm cultivation is facing serious constraints that are affecting negatively dates production. In the actual situation, the national demand of dates is partly satisfied by importation of important quantities that can reach sometimes 40 000 tons. To face this uncomfortable situation, Morocco has started in 2010 a novel strategy to resolve most of the constraints facing date palm sector development. In fact, a plant production program was launched to produce 3 million vitro-plants in the horizon of 2020. This program aims to rehabilitate actual oases (50 000 ha) and the creation of 17 000 Ha of new plantations. Organogenesis is the only technique used in this micropropagation program. Actually, eight private laboratories in collaboration with the National Institute for Agricultural Research (INRA) are in charge to produce these amounts of date palm plants. Till now, more than 1.8 million plants are already produced and the remaining amounts will be available before the stated date of 2020. The present paper gives an overview on date palm sector in Morocco and the role of micropropagation techniques in the framework of this important date palm multiplication program in Morocco.

Key words: Dates production, oasis, vitroplants, organogenesis, Bayoud disease.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is the main fruit crop in arid and semiarid regions, particularly in the arid regions of western Asia and North Africa. The palm tree is well adapted to desert environments that are characterized by extreme temperatures and water shortage, both in quality and quantity, due to scarcity of rainfall (Al-Yahyai and Manickavasagan, 2012). Date palm is considered the key species in the preservation of oasis ecosystems. Date palm cultivation is also one of the most economically important activity in the desert zones where it is cultivated not only for its valuable fruits but also to produce fuel, fibre and to provide shelter for ground crops. According to the World Food and Agricultural Organization, there are 105 million date palm trees worldwide. More than 60% of date palms are grown in the Middle East and North Africa. World production of dates is approximately 8.5 million metric tons corresponding to a cultivated area of about 1.4 million hectares (FAO, 2016).

In many countries, date palms suffer from serious biotic and abiotic constraints. Among theme, bayoud disease, a soil-borne fungus (*Fusarium oxysporum f. sp. albedinis*), is the most dangerous threat to date palm groves in North Africa. In this region, this disease has destroyed, since its appearance in 1780, more than 10 million palms in Morocco and 3 million in Algeria. It attacks particularly the most renowned cultivars which are very susceptible as Medjool, Deglet Noor, Boufegouss, etc.

Among the abiotic constraints, desertification is the most serious factor reducing palm groves in most of the countries where date palm is cultivated. This phenomenon is aggravated by prolonged drought periods conditions which hamper severely successful production of the crop. Taking into account those constraints, the need to select palms to replace senescent and destroyed date palm groves has been steadily increasing in decades. Hence, the exploitation of all available means of date palm propagation is required to fulfill the huge demand for palm vitro-plants.

DATE PALM SECTOR IN MOROCCO

Date palm cultivation areas are located on the southern flank of the Atlas Mountains (fig. 1). In terms of area, Morocco ranks seventh in the world, eleventh in terms of date palm numbers and twelfth in terms of date production. Dates production is around 111 000 tons annually (fig. 2). Regarding national consumption of dates, it varies from 3 kg/person as a national level to 15 kg/person in production areas (Sedra, 2015).

The actual number of date palm population is about 6.6 million of date palms which represents 6.2 % of the world's date population. It is composed of 453 cvs. represented by nearly 2.8 million trees (51.8 %) and approximately 2.6 million natural hybrids (khalts) (48.2 %). Only 45 % of date palms produce fruit each year, and the average productivity is around 20 kg/tree which is low compared to the standard date-producing countries (table 1). Almost 90 % of cultivation areas are located in three main oases in Ouarzazate, Errachidia and Tata provinces.

Date production ranks first among fruit crops in the oasis zones and contributes to 20–60% in the income of oasis farmers. The Green Morocco Plan (GMP), adopted by the Ministry of Agriculture since 2008, erected date palm cultivation as a priority to give new impetus to the agricultural economy in oasis areas.

DATE PALM SECTOR CONSTRAINTS

Date palm cultivation is confronted to many constraints that hamper the full expansion of this sector:

- Bayoud disease which had led to severe degradation of Moroccan palm groves mainly in terms of genetic erosion. About 2/3 of palm population have been destroyed during the last century. Hence, the cultivation area has been reduced from 150 000 hectares to less than 50 000 hectares actually.
- Climate aridity particularly long period of drought causing underground water resources degradation both in quantity and quality.
- Environmental degradation, mainly due to salinity, silting and desertification phenomenon in general.
- Insufficient infrastructure for processing, packaging and storing of dates under required standards conditions.
- Low and irregular productivity mainly due to the use of traditional farming techniques.
- Predominance of dates of low commercial value

  The direct consequences of all these constraints is that national palm groves are still poorly productive (in quantity and quality), with a dominance of genotypes of low market value and a production that does not covers the national demand for dates. As a result, Morocco is forced to import 40 000 tons during some seasons corresponding to 30% of national dates consumption (Bouziane, 2010).

DATE PALM SECTOR ASSETS

In spite of the aforementioned constraints, the oasis zones have considerable assets, able to attract national and foreign investments that can redress the situation of the date palm sector:

- The existence of a significant production potential in the sector, particularly in terms of improving the productivity, quality and added value of date palm products;
- The richness of the national gene pool in quality cultivars, such as the world-renowned Mejhoul variety, the most sought after in the world, which should be exploited judiciously for economic and commercial purposes;
- The existence of important local know-how that should be promoted as part of programs to improve farming techniques in the oases;
- The availability of national expertise and local know-how in the field of farming techniques that must be mobilized for technical assistance and palm groves development;
- The existence of investment opportunities through the extension of the date palm cultivation area and creation of modern palm groves in new zones free from Bayoud disease;
- The traditional character of oasis agriculture is in favor of the establishment of a biological date palm cultivation;
- Registration of the oases of southern Morocco in the Global Network of Biosphere Reserves of UNESCO in year 2000;
- The creation of a National Agency for the Development of Oasis Zones and the Argan Tree (ANDZOA) for the development of these regions (Bouziane, 2010).

OBJECTIVES OF DATE PALM SECTOR DEVELOPMENT PROGRAM

The Green Morocco Plan (GMP), adopted by the Ministry of Agriculture since 2008, erected date palm crop as a priority to give new impetus to the agricultural economy in oasis areas. The main axes of development of the date palm sector consigned in this plan can be summarized as follows (Anonymous, 2018):

- Providing investors and oasis producers with high quality date palm plants. National demand in this context has been estimated to be 3 million plants by the horizon of 2020;
- Increase of the date palm cultivation area by creating 17 000 hectares of new plantations in Bayoud disease free regions.
- Improvement of productivity and date production to reach 160 000 tons by the horizon of 2020;
- Improvement of the quality of the date production by the improvement of the profile of cultivated varieties;
- Valorization of the date production by the improvement of the marketed product in order to meet the requirements of national and international markets;

For the implementation of these objectives, a contract program was signed between the Moroccan government, the private laboratories responsible for the multiplication of date palm, the Inter-professional Federation of Dates and the National Federation of Date Producers in 2010.

Through its achievements in the fields of breeding, *in vitro* propagation, cultural techniques and dates processing, INRA has adhered to this program since its inception through collaboration and establishment of partnerships with the private sector to ensure the mass production of date palm vitroplants. This partnership has recently extended with the signing of two new agreements, bringing to 8 the total number of contracts linking INRA to private laboratories for the commercial multiplication of date palm.

DATE PALM MULTIPLICATION PROGRAM

The production of vitroplants of date palm is one of the priorities of the Morocco Green Plan. In fact, the use of rapid propagation techniques is the most effective way to meet the huge palm plants needed in this program (3 million plants by the horizon of 2020). In this context, INRA has been appointed for production of starting plant material (clusters of buds in the multiplication stage) from the main cultivars solicited by the growers. This production of initial plant material is considered the bottle neck for the commercial micropropagation of date palm by private laboratories in Morocco.

To honor its commitments, INRA, through its tissue culture laboratory in Marrakech, has undertaken an ambitious research program to improve the various date palm micropropagation techniques and their adaptation to main Moroccan cultivars. The expected objective is to develop a routine mass propagation process and to transfer this technology to private laboratories for strengthening the national capacity in terms of date palm plants production. In order to meet the growing demand for plant material from the 8 private laboratories, INRA has created a new national laboratory for date palm micropropagation at Regional Centre of Agricultural Research in Errachidia. At commercial level, the only micropropagation technique used in this program is the multiplication by organogenesis from offshoot shoot tips tissues.

Hence, organogenesis is a technique based on the exploitation of meristematic potentials of young leaves taken from offshoot shoot tips to develop vegetative buds that are used in the multiplication process. In addition this technique is well known for producing true-to-type plants. To do this, a distribution of tasks was carried out in such a way that INRA takes care of the research aspects and production of initial plant material and the private laboratories will ensure large-scale propagation and the mass production of vitroplants. The adopted production program for 2010-2020 is summarized in Table 1. The mentioned cultivars in this program are Mejhoul, Boufeggous, Nejda and Bouskri varieties and some selected genotypes.

In this context, the research program carried out in the field of date palm micropropagation by organogenesis technique has led to the following practical results:

- Development of a routine *in vitro* multiplication process for date palm micropropagation by organogenesis technique (Abahmane, 2017; 2011).
- Adaptation of this multiplication process to a wide range of varieties and date palm selected genotypes made of more than 40 genotypes including resistant varieties to Bayoud disease, susceptible with good fruit quality varieties and some selected genotypes as well as some selected pollinating males (Anjarne et al., 2005).
- Transfer of the developed micropropagation technology to private laboratories in the framework of agreements between INRA and these laboratories.
- Production of more than 100 000 clusters of buds from various Moroccan genotypes and their transfer to private laboratories for mass production of date palm vitroplants;
- The cooperation between INRA and private laboratories has so far permitted production of more than 1.8 million date palm plants.

CONCLUSION

The date palm micropropagation program in Morocco is one of the most important pillars of the national strategy for the development of the date palm chain. The joint efforts of all the stakeholders, whether from the public or private sector, made it possible to honor the commitments made for the first phase of this project 2010-2015 (production of 1.5 million plants). The program of the second phase 2015-2020 (1.4 million plants) is well underway and it is expected that this threshold will be reached well in advance before the expiry of the program contract of the Green Morocco Plan scheduled for 2020.

LITERATURE CITED

Abahmane, L. 2017. Cultivar-dependent direct organogenesis of date palm from shoot tip explants. p.3-15. In: J.M. Al-Khayri, S.M. Jain and D.V. Johnson (Eds), Date Palm Biotechnology Protocols, Vol. 1. Tissue Culture Applications. Humana Press. New York.

Abahmane, L. 2011. Date palm micropropagation via organogenesis. p.69-90. In: Jain S.M., J.M. Al-Khayri and D.V. Johnson (Eds), Date palm biotechnology, Springer Dordrecht Heidelberg London New York.

Al-Yahyai, R. and Manickavasagan, A. 2012. An Overview of Date Palm Production. p.3-12. In: A. Manickavasagan, M.M. Essa and E. Sukumar (eds), Dates Production, Processing, Food and Medicinal Values. CRC Press, London New York.

Anjarne, M., Abahmane, L. and Bougerfaoui, M. 2005. La multiplication *in vitro* du palmier dattier : un outil de développement des palmeraies dévastées par la maladie du Bayoud. Proc. Le Développement Agricole Durable des Systèmes Oasiens Seminar. Erfoud Morocco 08-10 March. p.80-83.

Anonymous, 2018a. Ministère de l'Agriculture, de la Pêche maritime, du développement rural et des eaux. 2018. www.agriculture.gov.ma/

Anonymous, 2018b. Salon des Dattes Erfoud Maroc. www.sidattes.ma

Bouziane, M. 2010. Programme de la filière dattière. Pack Info 89:38-39.

Food and Agricultural Organization. 2016. www.fao.org

Haddouch, M. 1995. Situation actuelle et perspectives de développement du palmier dattier au Maroc. Proc. Le palmier dattier dans l'agriculture d'oasis des pays méditerranéens Seminar. Elche, Spain 26-28 April. p. 63-79.

Sedra, M.H. 2015. Date Palm Status and Perspective in Morocco. p.257-323. In: JM. Al-Khayri, S.M. Jain and D.V. Johnson (Eds), Date Palm Genetic Resources and Utilization, Vol. 1, Africa and the Americas. Springer Dordrecht Heidelberg, New York London.

Tables

Table 1: Main data of the date palm sector in Morocco. Source: anonymous, 2018b

Indicators	Value/quantity	Observations
Total number of palms	6.6 million	Including new plantations
Total area (Hectares)	50 000	Mean density of 132 palms/ha
Number of varieties	453	51.8 % of the total number
Number of natural hybrids	2.6 million	48.2 % of the total number
Mean annual production of dates (metric tons)	111 000	Relatively low with only 30 % of dates of merchantable quality.

Table 2: Program of initial plant material (clusters of buds) production by INRA laboratories 2010-2020

Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Number of clusters buds	12000	18000	25000	40000	40000	52000	52000	52000	52000	52000	52000

Figures

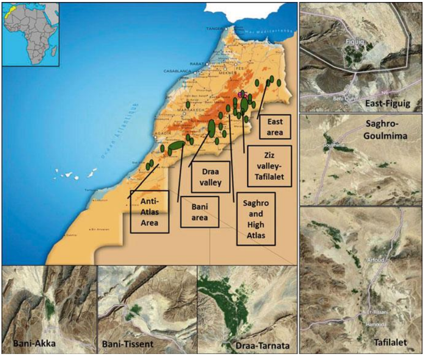

Fig. 1: Map of main areas of date palm cultivation in Morocco and some palm groves as seen from space. Source: Sedra, 2015.

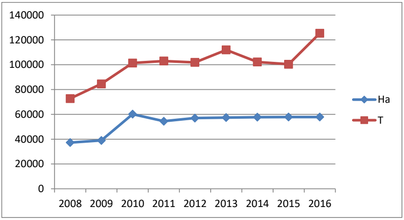

Fig. 2: Area (Hectares) and dates production (Tons) in Morocco from 2008 to 2016. Source: FAO, 2016.

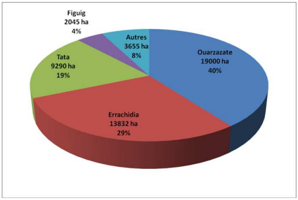

Fig. 3: Regional repartition of date palm areas in Morocco. Source: Haddouch, 1995.

Refined and Field Proven Micropropagation Technology for Commercial-scale Date Palm Plant Production

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Abstract

Micropropagation technology has been extensively applied for the mass production in different date palm cultivars worldwide. Organogenesis and somatic embryogenesis are the two methods used for the plant production. These two methods were manipulated by the type of explants and growth hormones during initiation. Millions of micropropagated date palms were produced by several laboratories using both methods and planted in the field since 1989 worldwide. The data collected through field evaluation during the past 25 years revealed the occurrence of several physiological disorders in the micropropagated date palms supplied by few commercial laboratories. Our study confirmed that many of the physiological disorders observed were common in both microrpopagated and offshoot propagated palms occurred due to the environment, pests and pathogens. Some of the physiological disorders such as dwarfing, rosettes of leaves, over production of offshoots, delayed flowering, abnormal flowers, pollen incompatibility and parthinocarpy were *in vitro* culture oriented due to the application of exogenous growth hormones in the culture media. In order to avoid these culture oriented physiological disorders, we undertook a research study on date palm micropropagation aiming to minimize or avoid growth hormones at different stages of micropropagation. Using the results obtained from our experimental study, we refined our date palm micropropagation technology. In the refined methodology, growth hormones were restricted to the initiation stage and completely avoided in all other stages of plant production. Somatic embryo desiccation, inverted culture technique, thin film liquid culture process and photoautotrophic culture system were developed and included at different stages of plantlet production instead of growth hormones application. Thousands of plantlets were produced through this refined technology and evaluated in the farms, and proved 100% free form the culture oriented physiological disorders. This refined micropropagation technology can be applied in commercial laboratories to get 100% customer satisfaction that will support the marketing of micropropagated palm trees.

Keywords: *Phoenix dactylifera*, growth hormones, physiological disorders, field evaluation.

INTRODUCTION

Clonal propagation of date palm (*Phoenix dactylifera* L) is traditionally carried out using the offshoots that are produced at the basal region of the tree during the initial vegetative growth phase. This method of propagation is slow, laborious, time consuming, expensive, susceptible to pest and pathogen infection, and low rate of field survival during transplantation. In order to overcome this issue, researchers had focused their attention towards the development of an alternative technology using plant tissue culture during 1970

Date palm tissue culture was initiated with little success in the year 1970 (Schroeder, 1970) and succeeded in developing protocols during late 1977 (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; Beauchesne, 1982). The culture media and protocol were 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. Latter on during the 21st century, the date palm micropropagation technology has been refined (Sudhersan et al., 2014; Elkosary, 2015) and much simplified for the commercial production. The senior author working on date palm micropropagation and tissue culture date palm field management (Sudhersan and AboEl-Nil. 2004) for the last 33 years has collected several information on date palm tissue, callus, embryos and plantlet responses to *in vitro* environment, and information about the field performances. Based on the 33 years of experience, we have refined the date palm micropropagation technology for commercial production. The details are presented in this report.

Explant Performance and Regeneration

All the tissue explant cultures kept under total darkness started expanding after a week from the date of culture initiation. The healthy explants after three-repeated sub-cultures in the same initiation media under dark started producing callus, somatic embryo primordia and shoot bud primordia. Callus developed mainly at the cut ends of the explants. Somatic embryo primordia and shoot bud primordia were developed on the leaf base tissue explants. When the explants transferred to the regeneration media under light, further growth and development occurred. The callus explants produced non embryogenic callus, somatic embryogenic callus and organogenic callus in the regeneration media after 2 subcultures. Explants with somatic embryo primordia developed in to somatic embryos directly. The explants with shoot bud primordia produced shoot buds directly. The somatic embryogenic callus produced somatic embryos when transferred to the hormone-free growth media. The shoot buds developed directly and indirectly, and somatic embryos developed directly and indirectly were regenerated into plantlets in the hormone-free culture media.

Growth Hormone Requirement

The plant growth hormone 2,4- dichlorophenoxy acetic acid (2,4-D) in the culture initiation media induced the tissue explants on callusing, somatic embryo production and shoot bud initiation. The media without 2,4-D failed to regenerate somatic embryos or shoot buds from the tissue explants. However, culture media without 2,4-D supported only the shoot tip cultures to regenerate multiple shoots under light. The growth hormone 2,4 D was mainly required only for the initial 30-60 days duration. The cytokinins were required for the proliferation embryogenic callus and organogenic callus. After the regeneration of embryogenic callus and organogenic callus, no growth hormones required for further plantlet production processes.

Multiplication and Hardening

Somatic embryo multiplication, embryo maturation, germination of somatic embryos, proliferation of shoot buds and plantlet growth occurred in the culture media free from any growth hormones. In the regeneration media containing cytokinins, somatic embryo maturation, normal plantlet regeneration and plantlet elongation were affected. The additional photoautotrophic culture system at the rooting stage supported 100% plantlets survived in the greenhouse. Hardened plants planted in the field showed uniform growth, early flowering and high yield. Few abnormalities were observed in the field which are common in seedling and offshoot derived palms due to the biotic and abiotic problems from the environment.

FIELD EVALUATION

Micropropagated date palm plantlets of 3 m, 6 m, 12 m and 24 m were planted in the field. Plantlets at the age of 12 and 24 m survived 100% without any difficulty in the field when they were planted in the month of March or October under the Kuwait's climatic conditions. The 3-6 m old plants in the open field needed much protection from the sand storm and high temperature for 100% survival. Field establishment took 6 m duration and once after the field establishment, all plants have grown and performed very well in the field without any protection during the summer months. After 24 months in the field, majority of the plants started producing the axillary shoots and during the 4th year of planting most of the palms started flowering. All the plants grown uniformly in the field. However, few growth abnormalities were noticed among the micropropagted palms.

In order to find out the real cause, the authors carried out investigations on seedling derived palms and offshoot derived palms in the field. Except few, many physiological disorders were found to be common among the 3 types of palms (TC derived, offshoot derived and Seedling derived). In order to study the difference between the palm trees produced by other commercial laboratories, we have planted TC derived palms of Barhi, Khlas, Madjool and Succary from two different commercial laboratories through local nurseries in the year 1995 along with our own TC palms. During the field evaluation, we have noticed few abnormalities on some of the palm trees obtained from other commercial labs such as excess of areal axillary shoot production (Rakoobs), pollen incompatibility, delayed flowering and parthinocarpy which were not occurred in our own TC palms. We are maintaining these palm trees for the last 25 years in our orchard for evaluation and data collection. As a surprise, all the abnormal palm trees turned to normal palms and started producing normal flowers and fruits after 18-20 years in the field. This study indicates that the abnormalities are culture oriented and may be due to the excessive application of growth hormones in the culture media.

The other physiological disorders such as Sudden dwarfing, crown bending, leaf whitening, frond malformation, terminal bud abortion, hapaxanthic axillary shoot production, "V" cut syndrome and rosettes of leaf production etc. are occurring commonly on all types of palms due to environmental factors. We have noticed that primary cause for the leaf abnormalities, dwarfing, "V" cut and crown bending are the larva of the greater date moth *Aphomia sabella* (Al-Shayji and Sudhersan, 2008; Sudhersan, 2013). All these physiological disorders caused from the environment can be controlled through identifying the real cause. From our experience on date palm micropropagation and field evaluation of micropropagated palms, certain abnormalities are due to the application of excessive growth hormones in the culture media. Through minimizing the hormone application, the reported abnormalities can be avoided. The application of growth hormone in the initiation media can not be avoided however, it can be reduced to a short duration (30-60 d). The somatic embryo maturation and germination, plant growth and elongation, rooting and hardening can be down through manipulating the culture environment. We have developed few techniques such as Desiccation, Thin film liquid culture, Inverted culture and Photoautotrophic culture for the date palm micropropagation to avoid growth hormones for embryo germination, plant growth, rooting and hardening.

Desiccation of Somatic Embryos

Somatic embryos when subcultured once in 20 days regularly multiplied continuously at non stop condition. Only 10 to 15 percent of the total embryos will be converted into plantlets and few will be vitrified. To stop the multiplicationand, vitrification, ant to enhance maturation and germination of somatic embryos, a stress has to be applied through chemicals in the culture media. We have developed a *in vitro* somatic embryo desiccation system which enhanced the percentage of somatic embryo conversion into plantlet from 15% to 90%.

Thin Film Liquid Culture

Thin film liquid culture technique has been developed instead of semisolid culture media for the fast plant growth. The plant growth and elongation in the semisolid culture media without growth hormone will be very slow and takes 60-90 d to reach the rooting size plantlets. A liquid media having half strength MS basal salts with organic additives and low concentration of sucrose was developed and used after the somatic embryo germination. Only 2-3 ml liquid media was poured in each test tube to just make the base of the explant to touch the medium. All the small plantlets produced by somatic embryos were transferred to the test tubes containing 3 ml of liquid media showed fast growth when compared to the semisolid culture media and plantlets reached the rooting stage within 20-30 d.

Inverted culture technique

When the plantlets are cultured in normal way, the basal part of the plantlets will be immersed inside the culture media. This causes vitrification and production of more axillary shoots. In order to desiccate the stem bas and fast plant growth and root initiation, we developed inverted culture technique. Under this method, small plantlets were planted upside down in the thin film liquid media and also in the semisolid culture media. Only the leaf tip will touch in both types of media. The plantlet elongation was faster and also the stem became more stronger (Figs. 1-3). Majority of the plantlets under this culture method showed the adventitious root initiation.

Photoautotrophic Culture

The micropropagated plants were not very strong to face the outside climatic conditions due to the undeveloped stomata, less cuticle on the leaf surface and poor root system. Therefore, in order to make the plantlets stronger before transferring to the greenhouse and to stop the plant mortality during hardening, photoautotrophic culture system was developed. The culture media was prepared by using sterile potting soil mixed with half strength MS liquid media without any growth hormone and sugar. The elongated plantlets (10-15 cm length) at the stage of adventitious root initiation stage were transferred to photoautotrophic culture media, and maintained under high light intensity (5000 Lux) in the culture rooms for growth and elongation. In the photoautotrophic culture media, plantlets were more hardened and the root system with more capillary roots developed. The leaves turned dark green in cooler with well developed chloroplast. The surface of the leaves produced more waxy coating during this photoautotrophic culture phase. The plantlets produced through this photoautotrophic culture system survive 100% in the greenhouse.

Refined Micropropagation Technology

Through our research and development and 30 years of research experience on date palm micropropagation and field evaluation of TC plants, we have refined the date palm micropropagation protocol for commercial production. In our system, growth hormones were used only for culture initiation stage and the hormones were totally avoided in all other stages of micropropagation. The growth hormone applications in the culture media was avoided after the shoot regeneration (organogenesis) or somatic embryo regeneration (Somatic embryogenesis). Somatic embryo maturation and germination, vitrification control, tissue browning control, plantlet elongation, root induction, development of strong plantlets with well developed chloroplast, cuticle and root system are being carried out without using any growth regulators. Techniques such as desiccation of somatic embryos, thin film liquid culture integrated with inverted culture technique, manipulation of temperature and duration of light and dark, photoautotrophic culture system and a new sugar and hormone-free photoautotrophic culture media. We have produced a large number of plantlets through tissue culture methods (both organogenesis and somatic embryogenesis) using our refined technology without any genetic disorders or somaclonal variations. Thousands of plants are being grown in KISR research station as well as in client farms. There is no genetic malformation observed and reported by our clients who has took TC date palms from our laboratory (Figs. 4,5).

CONCLUSIONS AND RECCOMMENDATIONS

Our study concluded that date palm can be micropropagated through organogenesis or somatic embryogenesis methods. The auxin 2,4-D is necessary for the induction of somatic embryogenesis from tissue explants and a strong cytokinin is required for the induction of organogenesis. After the initiation of shoot buds or somatic embryos, there is no need for growth hormones. The palm trees produced both methods of regeneration through our method are field proven for true-to-type clonal nature without any genetic abnormalities. Somatic embryogenesis is more cost effective method of plant production than the organogenesis method. Date palm can be micropropagated through different media with different combinations and concentrations of growth hormones. Excessive growth hormone application in the date palm micropropagation system will cause severe economic loss to the farmers. Therefore, with our 30 years of field experience on TC date palms, we recommend the following to make customer satisfaction on TC pals;

1. Use the main shoot meristem for organogenesis.
2. Use leaf primordial explant for somatic embryogenesis.
3. Do not use any growth hormones in the culture media after the induction of shoot buds or somatic embryos.
4. Carry out subcultures once in 15-20 d and do not maintain the cultures for long time (more than 30 days continuously) in the culture media.
5. Try to avoid floral explants for true-to-type clonal micropropagation. If it is unavoidable use the floral primordia before the occurrence of gametogenesis.
6. Use offshoots from healthy and well known mother palm trees.
7. Test the plants in the field prior to marketing.
8. Do not apply any growth hormones for hardening and plant growth in the greenhouse or nursery.

ACKNOWLEDGEMENT

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LITERATURE CITED

Al-Shayji Y. and Sudhersan, C. 2008. Pseudodwarf disorder control in tissue cultured date palms. Amer. Eur. J. Sci. Res. 3 (2): 128-131.

Ammar, S. and Benbaids, A. 1977. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by tissue culture of young plants derived from seeds. Compte Rendu Hebolomadaires de l'Acad. des Sci. D. 28 : 1787-1792.

Beauchesne, G. 1982. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by *in vitro* cultures. Proc. 1st Symposium on Date Palm. King Faisal University, Saudi Arabia. p. 698-699.

Drira, N. and Benbadis, A. 1985. Vegetative Multiplication of Date-Palm (Phoenix dactylifera L.) by Reversion of *In Vitro* Cultured Female Flower Buds. J. Plant Physiol. 119: 227-235.

Elkosary, S. 2015. Date Palm Micropropagation. https://www.researchgate.net/publication/275969474

Reuveni, D. 1979. Embryogenesis of plantlet growth of date palm (*Phoenix dactylifera* L.) derived from callus tissue culture. *Plant Phys*iology 63: 138 (Abstract).

Schroeder, C.A. 1970. Tissue culture of date shoots and seedlings. Rep. Ann. Date Growers' Institute 47:25-27.

Sharma, D.R., Sunita, D. and Chowdhury, J.R. 1986. Regeneration of plantlets from somatic tissue of the date palm (*Phoenix dactylifera L.)*. Indian J. Exp. Biol. 24: 763-766.

Sudhersan, C., AboEl-Nil, M. and Al-Baiz, A. 1993. Occurrence of direct somatic embryogenesis on the sword leaf of in vitro plantlets of *Phoenix dactylifera* L, cultivar Barhee. *Current Science* 65:877-879.

Sudhersan, C. 1989. Morphological and developmental studies on embryos and seedlings of some palms. Ph.D Thesis, Madurai Kamaraj University, Madurai, India.

Sudhersan, C., and M. AboEl-Nil. 2004. Axillary shoot production in micropropagated date palm. *Current Science* 86: 771-773.

Sudhersan, **C.** 2013. Date palm cultivar specific susceptibility to grater date moth infestation. American-Eurasian Journal of Sustainable Agriculture, 7:32-36.

Tisserat, B. 1979. Propagation of date palm (*Phoenix dactylifera* L.) in vitro. J. of Exp. Botany 30: 1275-1283.

Tisserat, B and De Masion, D. 1980. A histological study of the development of adventive embryos in organ cultures of *Phoenix dactylifera* L., Ann. Bot. 46: 465-472.

Ziad, A. and P. F. De Wet. 2002. Date palm propagation. In: A. Zaid (eds), Date palm cultivation, FAO Plant Production and Protection Paper No. 156, Rome, Italy.

Figures

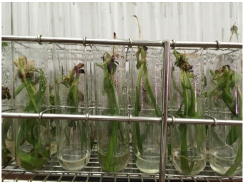

Fig. 1. Inverted thin film liquid culture **Fig. 2**. Inverted culture **Fig.3**. Root initiation


Fig. 4. Large-scale TC date palm production **Fig. 5.** TC palms in the field showing uniformity

Conform and healthy tissue culture propagation of date palm

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Abstract

Due to its economic and social importance in many desert areas extending from Western North Africa to India, Date Palm (Phoenix dactylifera) is multiplied by tissue culture in order to eliminate pathogenic agents (fusarium oxysporum f.sp.albedinis, endogenous bacteria …). In vitro somatic embryogenesis methodology, used by a lot of tissue culture laboratories, usually induces specific bacterial contaminants according to the size of the central meristematic zone used to start the embryogenesis callus. Our study consisted in: Preserving as well as possible the true-to-type conformity for microprogation of different date Palm varieties; Obtaining cultures free of endogenous pathogenic agents; and Using a non-destructive process. Tissue cultures were initiated from both axillary meristems and immature inflorescences and micropropagated by organogenesis method. Five types of apex were used and propagated in aseptic conditions during the three stages of micropropagation (initiation, multiplication and rooting). The health status was verified by electron microscopy INRA studies. No bacterial contaminants were observed in tissue cultures obtained by one type of apex and by immature inflorescences, which confirms the possibility to multiply by tissue culture true to type date palm without endogenous pathogenic agents.

Keywords: Date Palm, contaminants, meristem tip culture, *in vitro* propagation, health regeneration.

Enhanced in vitro multiplication and rooting of date palm cv. Yellow Maktoum by zinc and copper ions

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Abstract

The present work was carried out to study the effect of $ZnSO_4$ and $CuSO_4$ on the in vitro multiplication stage during two types of subculture intervals (4 and 6 weeks) and rooting stage of date palm cv. Yellow Maktoum. The pigment content have been estimated (chlorophyll a, b, total chlorophyll and carotenoids), protein and proline contents during multiplication stage from this study. Blending have been microelements compounds $ZnSO_4$ or $CuSO_4$ separately at different concentrations (0.0, 10.0, 25.0. 50.0. 75.0. 100.0 µM) to a Murashige and Skoog (MS) medium as a basal nutrient medium with recommendation of auxins and cytokinins during both multiplication and rooting stages. After three subculture from culturing on the same concentration of $ZnSO_4$ and $CuSO_4$ data showed that, $ZnSO_4$ at 50 µM or $CuSO_4$ at 25 µM induced a maximum morphogenic responses (number of shoots, number of secondary embryos, shoots length (cm) growth vigor/expaln) and physiological responses (Chl a, b, total Chl, Car content and total protein content) during the two subculture intervals under investigation. Further, proline content increased by increasing $ZnSO_4$ or $CuSO_4$ concentrations in MS medium. Regarding the subculture interval, usually 6 weeks was better than 4 weeks of all measurements. Otherwise, The best treatments for the in vitro rooting were the strength MS basal nutrient medium supplemented with $ZnSO_4$ at (75 µM or 100 µM) or $CuSO_4$ at (50 µM or 75 µM) whereas, number of roots, roots length, plantlets length(cm) and growth vigor/explant achieved a maximum values with that treatments. All rooted plantlets were transferred to green house for acclimatization. These plantlets achieved 90% survival rate after 6 months from culturing in greenhouse. The present study was aimed at standardizing the nutrient requirements for improved micropropagation of date palm cv. Maktoum during multiplication and rooting stages by manipulating zinc and copper levels in MS medium for obtaining full plantlets with good root system are able to resume their development successfully at acclimatization.

Keywords: Date palm, Carotenoids, $CuSO_4$, In vitro, Multiplication, proline content, Rooting, Subculture interval, total chlorophyll, total protein and, $ZnSO_4$

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is a perennial, dioecious, and monocotyledonous tree well adapted to arid environments. Typically, date palm propagation is sexually by seed or vegetatively by offshoots. However, both techniques are economically inefficient and fail to meet the demand for large quantities of planting material and the clonal propagation of selected superior genotypes (Al Khayri 2007 and Aahmane 2017). There have been previous reports on date palm micropropagation through the organogenesis and somatic embryogenesis (Fki et al., 2011;Zayed 2017). Although the great achievement in date palm *in vitro* propagation there are still serious problems during its reproduction cycle inside lab which may be defused or stop the successfully transferring to green house. This work presents noticeable problem during multiplication stage as the converted plantlets (resulted from somatic embryos differentiation) did not pass to rooting stage in good manner of growth due to their shortness ,weakness and low number of multiplied shoots ,so that may decrease their opportunity for successful transfer to acclimatization stage. Inorganic macronutrient and micronutrient levels used in most plant tissue culture media are based on levels established by Murashige and Skoog (1962) for tobacco tissue culture. However, many plant species and varieties do not respond to classical approach, demonstrating that alterations in hormonal ratios cannot be the sole mechanism controlling in vitro developmental processes (Ramage and Williams 2002).

Zn and Cu are micronutrients of growth medium that are needed by plants for growth and various biochemical and physiological pathways (Narula and Srivastava 2005). Zn plays a vital role in the cell division, cell expansion, proteins synthesis, and also in carbohydrate, nucleic acid and lipid metabolism. Zn is required for the synthesis of tryptophan (Tsonev and Lidon 2012), which is a precursor of IAA this metal also has an active role in the production of auxin, an essential growth hormone (Brennan 2005).zinc increases the biosynthesis of chlorophyll and carotenoids (Broadley et al., 2007).

Copper (Cu) is an important part of enzymes and protein involved in plant metabolic processes such as photosynthesis and mitochondrial electron transport. It is an essential micronutrient required for proper plant growth and development (Shahid, et al., 2015). Copper is a micronutrient important for normal plant growth and development. It takes part in processes of photosynthesis, respiration, transport and other physiological and biochemical functions (Yruela, 2005). In many plant species increasing of copper level in culture medium has a positive effect on *in vitro* regeneration, elongation and micropropagation (Bardar et al., 2014). Therefore, optimum Cu and Zn concentrations in the medium positively affect development of the membrane system of chloroplasts and chlorophyll content. Proline accumulation is reported to occur in response to heavy metal toxicity (Sharma and Dietz 2009 and
Ahmed et al., 2015).

The present study was aimed at standardizing the nutrient requirements for improved micropropagation of date palm cv. Maktoum during multiplication and rooting stages by manipulating zinc and copper levels in MS medium for obtaining full plantlets with good root system are able to resume their development successfully at acclimatization.

MATERIALS AND METHODS

This experimental work was performed at the Central Laboratory of Date Palm Researches and Development Giza, Egypt. Date palm off shoots cv. Yellow Maktoum have been received from Iraq country under suppression of Dr. Zeinab Zayed to propagate this cultivar under Egyptian condition. Sterilization protocol for meristematic shoots tip were performed according to (Zayed, 2017).

Effect of zinc sulphate ($ZnSO_4 . 7H_2O$) and copper sulphate ($CuSO_4 . 5H_2O$) on multiplication stage.

Plant material: Explants material in this stage were shoots cluster multiplied consist of (4-5shoots at 0.5-0.7 cm length) which received by indirect somatic embryogenesis protocols according to (El-Dawayati et al., 2014).

Medium component: MS (Murashige and Skoog 1962) salt strength medium and vitamins were used as a basal nutrient medium, supplemented with 40 gl^{-1}sucrose, 0.54 µM NAA and 0.222 BA µM (control treatment). Studied levels of zinc sulphate ($ZnSO_4 . 7 H_2O$) and copper sulphate ($CuSO_4 . 5 H_2O$) were added separately to nutrient medium at different concentrations 0.0 (control treatment, having normal zinc or copper concentration in MS basal medium), 10.0, 25.0, 50.0, 75.0 and 100.0 µM. All the salts used were of analytical laboratory. The pH of the medium was adjusted to 5.7± 0.1 before adding bacteriological grade agar (*Qualigens*) at 8 gl^{-1} and medium was dispensed into small jars 150 ml (40ml/jar) before autoclaving at 121ºC and 1.1 kg/cm^2 for 20 min. Culture jars of each treatment were divided to three replicates. Each replicate consists of three culture jars .Each jar contained one of shoot cluster explant. All culture jars of each treatment were incubated under 100µmol/ m^2/s provided by florescent lamps for 16 and 8 hrs. dark at 25 ± 2ºC. Data were recorded after three subcultures during the two subcultures intervals 4 weeks and 6 weeks about morphogenic responses (shoots number, number of secondary embryos formation, shoots length (cm) and growth vigor/ explant) and physiological responses (chlorophyll a, b, total chlorophyll, carotenoids content and total protein content). Proline content was determined to indicate the effect of using high concentrations of $ZnSO_4$ and $CuSO_4$ as a sign of stress.

The data of growth vigor/ explant were scored visually according to Pottino 1981; Mujib et al., 2005 ; Zayed, 2014 as follows:-

1-Negative result (-)
2-Below average result (+)
3-Average result (++)
4-Good result (+++)
5-Very good result (++++)

The pigments were determined (mg g^{-1} FW) using the method described by Arnon (1949). Determination of protein content mg g^{-1} FW of leaves was assessed by the method described by Bradford (1976). The proline content mg g^{-1} FW of leaves was determined by Bates et al., (1973)

Effect of zinc sulphate ($ZnSO_4$) and copper sulphate ($CuSO_4$) on rooting stage

The same concentrations of $ZnSO_4$ and $CuSO_4$ were studied during rooting stage, subculture interval in this testing stage will detriment according to the results obtain from multiplication stage.

Plant material: Explant material in this stage were elongated shoots (shootlet) 7 cm about with 2 leaves were excised from elongated shoots received from microprpagation protocols according to (Zayed 2017).

Medium component: medium components in the rooting studying of date palm cv. Yellow Maktoum are the same components above mentioned during multiplication studying except sucrose concentration is 50 gl^{-1} and growth regulators are 1.36 µM paclobutrazol (PBZ), 5.37 µM NAA and 4.92 µM IBA (Zayed 2017). Culture tube (2.5 x 25 cm) containing 20 ml rooting medium adding to different concentrations of zinc sulphate ($ZnSO_4$. 7 H_2O) and copper sulphate ($CuSO_4$. 5 H_2O) separately as mentioned above. Culture tubes of each treatment were divided to three replicates. Each replicate consists of three culture tube .Each tube contained one shootlet explant.

All culture tubes of each treatment were incubated under 200µmol $m/^2$ / s provided by florescent lamps for 16 and 8 hrs. dark at 27 ± 2ºC. Data were recorded after three subcultures about shoot length/shootlet, root number/shootlet, root length/shootlet (cm) and growth vigor/shootlet which described above mentioned.

Rooting and acclimatization

Elongated shootlets which were received from all previous treatments of multiplication experiment were collected and cultured on rooting medium consisting of 1/2 MS basal nutrient medium supplemented with 5.37 µM NAA, 4.92 µM IBA and 1.36µM paclobutrazol (PBZ) and then, the shootlets were separated to individual shoots and cultured on preacclimatization medium (Zayed 2017).

All rooted plantlets from either multiplication or rooting experiments were transferred to liquid preacclimatization medium composed of 1/4 MS medium containing $10.0g^{-1}$ sucrose and $6g^{-1}$ polyethylene glycol 8000 (PEG). Plantlets with well developed shoot and root system were carefully transferred to pots containing peatmos : vermiculate: sand 1:1:1 after it washed with tap water. Humidity was maintained initially by covering the pots with transparent polythene bags.

Statistical analysis

The factorial design in completely randomized arrangement was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% probability level according to Snedecor and Cochran (1972).

RESULTS

In the present study, date palm cv. Yellow Maktoum were grown in vitro and the effect of different levels of $ZnSO_4$ and $CuSO_4$ were assessed. Analysis of Variance (ANOVA) showed significant effect ($P \leq 0.05$) for $ZnSO_4$, $CuSO_4$ treatments and subcultures intervals for all measurements during multiplication and rooting stages.

Effect of zinc sulphate ($ZnSO_4$) on multiplication stage

After three subculture from culturing shoots cluster explants of date palm cv. Yellow Maktoum on different levels of $ZnSO_4$ under two subculture intervals (4 weeks and 6 weeks). The control MS medium supplemented with 0.222 µM BA and 0.54 µM NAA (recommended medium during multiplication stage) reproduction 14.75 shoots/explant after 4 weeks and 18.25 shoots /explant after 6 weeks. The addition of different levels of $ZnSO_4$ from 10.0 to 100.0 µM was optimization of shoots number healthy compared with control medium. $ZnSO_4$ at 50.0 µM was good result of number of shoots which recorded 36.12 shoots/explant. There are significant differences between subcultures intervals (4 and 6 weeks) of the shoots numbers produced where 6 weeks were the perfect than 4 weeks (25.70 shoots /explant and 24.30 shoots /explant respectively) Table 1. On the other hand interaction between different levels of $ZnSO_4$ and subculture intervals had significant effect of shoots number/explant. Cluster explants when cultured on $ZnSO_4$ at 50.0 µM for three subcultures and subculture intervals were 6 weeks produced the superior of shoots number (40.0 shoot/explant) during multiplication stage.

The number of secondary embryos formed on the bases of the cluster explants was also evaluated during two subculture intervals under investigation Table 2. It was found that 50.0 µM $ZnSO_4$ promoted secondary embryos formation. The higher concentrations of $ZnSO_4$ (75.0 and 100.0 µM) reduced secondary embryos formed (8.37 and 3.72 secondary embryos/explant respectively).

Concerning subculture intervals, there wasn't significant differences between secondary embryos number formed after 4 weeks or 6 weeks during multiplication stage (7.07 and 8.05 secondary embryo/explant respectively). Interaction effect between treatments studied and subculture intervals clearly affected significantly of the secondary embryos numbers formed on the bases of the cluster explants during multiplication stage, cluster explants cultured on 50.0 µM $ZnSO_4$ showed increasing in the secondary embryos number formed with 6 weeks as a subculture interval.

The effect of different levels of $ZnSO_4$ and subculture intervals on shoots length (cm) was studied during multiplication stage in Table 3. The results showed that $ZnSO_4$ at 50.0 and 75.0 µm gave the longest shoots without significant differences in between (6.87 and 6.12 cm /explant respectively). The subculture interval every 6 weeks was better than 4 weeks of shoots length. Regarding interaction effect between different levels of $ZnSO_4$ and subculture intervals of shoots length (cm) data indicated that $ZnSO_4$ at 50.0 or 75.0 µM and 6 weeks as a subculture interval was optimum of shoots length (7.5 cm/explant) during multiplication stage.

From observation in Table 4 cluster explants cultured on $ZnSO_4$ at 50.0 or 75.0 µM showed the highest significant results in growth vigor signs during multiplication stage (3.87 and 3.62 /explant respectively) without significant differences in between, best signs of growth vigor appeared in strong shoots and well green color without marks of browning or welting. Where cluster explants cultured on high concentration of $ZnSO_4$ at 100.0 µM showed bad marks of growth as browning and weak shoots. The interaction effect between different levels of $ZnSO_4$ and subculture intervals on growth vigor clusters explant, data observed that $ZnSO_4$ at 50.0 µM after 6 weeks was the best of growth vigor (4.0) during multiplication stage.

The Chl *a*, Chl *b*, and total Chl content in the multiplication in vitro of date palm cv. Yellow Maktoum increased with the increasing zinc concentrations up to the level 50.0 µM $ZnSO_4$. Higher concentrations of $ZnSO_4$ caused a decline in the photosynthetic pigments. While the Car content increased up to level of $ZnSO_4$ 25.0 µM and declined thereafter. Regarding the subculture interval, 6 weeks was better than 4 weeks of all pigments content (Fig. 2).

Zinc is an essential micronutrient and plays an important role in protein synthesis, enzyme activation and growth regulation, so data presented in Fig 3. indicated that addition $ZnSO_4$ at 50.0 µM to date palm multiplication medium increased significant in total protein content after 6 weeks as a subculture interval (1.6 mg/g FW) whereas that, $ZnSO_4$ at 75.0 µM achieved the same value of total protein content (1.6 mg/g FW) after 4 weeks. The level of ZnSO4 above 100.0 µM reduced total protein content of date palm shoots during multiplication stage.

Results showed that proline accumulation increased significantly by increasing $ZuSO_4$ concentrations from 10.0 to 100.0 µM and do not significant differences of proline accumulation between two type of subculture interval (4 and 6 weeks) Fig.4.

Effect of copper sulphate ($CuSO_4$) on multiplication stage

Moreover, studies were conducted with modified levels of copper in the multiplication medium of date palm cv. Yellow Maktoum for three subculture under two type of subculture intervals (4 and 6 weeks). The addition of $CuSO_4$ at the concentration from 10.0 to 100.0 µM was beneficial for production of shoots and their growth as compared to the control treatment (having normal copper concentration in MS basal medium). There was concomitant increase in number of shoots with increasing concentration of copper till the level for mineral is optimized at 50.0 µM which achieved 40.50 shoot / explant after 6 weeks of culture. In addition to that increase in copper concentrations had adverse effect on number of shoots formed /explant. The subculture intervals 6 weeks was the perfect than 4 weeks of shoot number formed /explant (30.25 and 24.12 / explant respectively) with high significant differences in between (Table 5).

The results in Table 6 showed that the addition of $CuSO_4$ at 100.0 µM for three subcultures to multiplication medium of date palm cv. Maktoum depressed the formation of secondary embryos to give the lowest significant results both of subculture interval under investigation (4.45 and 6.75 secondary embryo/explant respectively). Where the highest induction of secondary embryos/explant was observed with $CuSO_4$ at 10.0 and 25.0 µM in formula of MS nutrient salts (12.50 and 11.25 secondary embryo/explant respectively) after 6 weeks without significant differences in between. The high efficiency of secondary embryo production ensures the production of large numbers of explants for shoot regeneration.

As regards shoots length (cm), the addition of $CuSO_4$ at 50.0 µM to culture multiplication medium achieved the highest significant value in increasing shoots length/ cluster explants (6.97cm) during multiplication stage (Table 7). On the other hand cluster explants cultured on the high level of $CuSO_4$ (100.0 µM) of two types subculture interval under investigation gave the lowest significant results in increasing shoots length (3.75 and 5.50 cm /explant). There was significant effect of subcultures intervals on shoots length cluster explants during multiplication, increasing of subculture interval of cluster explant cultured on different levels $CuSO_4$ from 4 weeks to 6 weeks achieved the longest shoots. Data showed that increasing in shoot length for all cultured explants during multiplication stage had affected significantly with interaction between studied levels of $CuSO_4$ and the subculture intervals (4 and 6 weeks) .This was improved clearly with cluster explants cultured on $CuSO_4$ at 50.0 µM by means of 6 weeks(7.50 cm /explant).

Clearly from data in Table 8 clusters explants culture on different levels of $CuSO_4$ in formula of MS from 10.0 to 75.0 µM nutrient salts showed the highest significant results in growth vigor signs during multiplication stage (3.25, 3.87, 3.78 and 3.37 /explant respectively).The best signs of growth vigor appeared in strong shoots and well green color without marks of browning or welting. Where cluster explants cultured on high concentration of $CuSO_4$ seemed bad marks of growth and visible symptoms of toxicity were observed especially at the 100 µm Fig. 5. The interaction effect between all different levels of $CuSO_4$ and intervals subcultures showed big impact on growth vigor/cluster explants. Where the highest significant value of growth vigor/cluster explants was recorded the same value after 6 weeks on $CuSO_4$ at 25 and 50 µM (4.0).

The Chl *a*, Chl *b*, total Chl and car content in the multiplication in vitro of date palm cv. Yellow Maktoum increased with the increasing copper concentrations up to the level 50.0 µM $CuSO_4$. Higher concentrations of $CuSO_4$ caused a decline in the photosynthetic pigments. Concerning the subculture interval effect, there wasn't significant differences between 4 and 6 weeks of all pigments content (Fig. 6).

Concerning the effect of different levels $CuSO_4$ and two types of subculture interval on total protein content where cu is an important part of protein involved in plant metabolic processes such as photosynthesis and it is an essential micronutrient required for suitable plant growth. Clearly from data in Fig. 7 showed that low concentrations of $CuSO_4$ (10.0 and 25.0 µM) encouraged increasing significant of total protein content compared with the higher concentrations (50.0, 75.0, 100.0 µM) both of the subculture interval under investigation (4 and 6 weeks). With regard to the effect of subculture interval on total protein content during multiplication stage of date palm, 4 weeks was the perfect than 6 weeks of total protein content.

The effect of different concentrations of $CuSO_4$ and subculture interval on proline content was recorded in Fig. 8, the proline accumulation increased significantly by increasing $CuSO_4$ concentrations and the maximum proline content of 0.77 or 0.78 mg g^{-1} (fw) was on MS medium containing either 75.0 or 100.0 µM $CuSO_4$ without significant differences in between. On the other hand, there aren't significant differences in between 4 weeks and 6 weeks as a subculture interval on proline content

Effect of zinc sulphate ($ZnSO_4$) on roots growth

Shootlet derived from somatic embryos (5 - 7 cm shoot length and 2 leaves/plantlet) were cultured on rooting medium of date palm cv. Maktoum supplemented with different concentration of $ZnSO_4$ (0.0, 10.0, 25.0, 50.0, 75.0 and 100.0 µM) in formula of MS nutrient salts in order to enhance and encourage root formation Fig. 9.

Data in Table 9. showed clearly effect different concentrations $ZnSO_4$ on root growth of shootlets date palm cv. Maktoum for three subcultures. The addition of $ZnSO_4$ with different concentrations from 10.0 to 100.0 µM to rooting medium was beneficial to form good root system compared to the control treatment (having normal zinc concentration in MS basal medium).
The high concentrations of $ZnSO_4$ promoted roots growth, where $ZnSO_4$ at 100.0 µM was the superior concentration of shoots length (12.5 cm/explant), number of roots (8.1/explant) and root length (8.0 cm/explant) comparative with control medium and other treatments. Further the shootlets which cultured on rooting medium containing $ZnSO_4$ at 100.0 µM appeared stronger shootlets and well green color without marks of browning or welting as the best sign of growth vigor of shootlets explants (4.0 /shootlet explant).

Effect of copper sulphate ($CuSO_4$) on roots growth

Data in Table 10 showed the effect of copper sulphate ($CuSO_4$) on root growth during rooting stage of date palm cv. Yellow Maktoum for three subcultures. The addition of $CuSO_4$ at 75.0 µM in formula of MS nutrient salts achieved the highest significant value of shoots length (13.4 cm/explant) and number of roots (9.2 root/explant) after three subcultures from culturing. While either concentrations 25.0 and 50.0 µM of $CuSO_4$ were the optimum concentration of root length without significant differences in between (8.3 and 8.6 cm /explant respectively). As regard growth vigor of shootlets date palm cv. Yellow Maktoum during rooting stage, data clearly observed that growth vigor as a sign of stronger shootlets increased with increasing $CuSO_4$ level in formula of MS nutrient salts from 10.0 to 100.0 µM (2.4, 3.2, 3.8, 3.8, 4.0 and 4.0/shootlets explant respectively)(Fig. 10)

Generally, the highest concentrations of $ZnSO_4$ (100.0 µM) and $CuSO_4$ (75.0 µM) were the best concentrations of all growth parameters during rooting stage in vitro (shoots length, number of roots, roots length and growth vigor) to produce optimize plantlets able to transfer acclimatization stage successfully**.**

Eventually**,** all plantlets have been received from using $ZnSO_4$ or $CuSO_4$ at the certain best treatments during multiplication stage were accelerated in growth during rooting stage as well as the all plantlets have been received from using $ZnSO_4$ or $CuSO_4$ at the certain best treatment during rooting stage were collected and transferred to preacclimatization liquid medium. All rooted plantlets were transferred to green house for acclimatization with maintaining high humidity by covering transparent polyethylene bags. These plantlets achieved 90% survival rate after 6 months from culturing in the greenhouse. Thus our results promote the process of date palm micropropagation

DISCUSSION

In the present study, date palm cv. Yellow Maktoum was grown in vitro and the effect of different levels of $ZnSO_4$ and $CuSO_4$ were assessed during multiplication and rooting stages after three subcultures. We observe here that manipulating the salt strength might also modify the growth of plantlets since a suitable salt strength may work as important elicitor of in vitro morphogenesis. Furthermore, cell growth and morphogenesis of some species may be enhanced by increasing levels of mineral salts above those recommended by Murashige and Skoog (1962). Zinc is an essential micronutrient of growth medium that is needed by plants for growth and various biochemical and physiological process such as protein synthesis, enzyme activation and growth regulation and the maintenance of membrane structure. It has been confirmed by our results whereas, there was concomitant increase in all growth parameters (number of shoots, number of secondary embryos, shoots length (cm), growth vigor) with increasing concentration of zinc till the level for mineral is optimized at 75.0 µm and thereafter decreased. The positive role of $ZnSO_4$ has been documented earlier by Ali et al., 1999 in *Bacopa monniera*, Sharma et al., 2010 in *Pisum sativum* L. *Cicer arietinum*, Fatima et al., 2011 in *Withania somnifera* and El-Jassani 2013 in *Phoenix dactylifera*. Also Shahid et al. 2015 declared that, optimized MS medium supplemented with different concentration of $ZnSO_4$ was better than control with respect to multiple shoot induction and also length of shoots. Medium containing $ZnSO_4$ (100 µM) induced maximum number (5.20 ± 0.37) of shoots with shoot length (0.80 ± 0.027 cm). Similar stimulatory effect of zinc in MS basal nutrient medium has been reported by Ahmed et al., 2015 who said, incorporation of 25 µM $ZnSO_4$ to a Murashige and Skoog (MS) medium with optimized concentrations of auxins and cytokinins induced a maximum number of shoots per explant (45.47 ± 0.24) in *Rauvolfia serpentina*. The level of $ZnSO_4$ above the optimum prove to be inhibitory for shoots formation. In this study, the Chl *a* Chl *b* and Car increase up to the optimal Zn concentration (50.0 µM) and thereafter decreased. High concentration of $ZnSO_4$ (100.0 µM) inhibited Chl total and Car content this result is in accordance with the earlier reports like Khudsar et al., 2004 in *Artemisia annou*, Sharma et al. 2010 in *Pisum sativum* and Ahmed et al., 2015 in *Rauvolfia serpentine*.

Our results proved that, the optimum level of $ZnSO_4$ (50.0 Mµ) increase total protein content but above 100.0 Mµ decrease total protein content of date palm shoots during multiplication stage, these results are line with Ali et al., 2000; Hansch and Mendel 2009; Tsonev and Lidon 2012 who reported that, Zn is implicated in protein synthesis and energy production. It is also involved in nucleic acid synthesis, carbohydrate and lipid metabolisms. Proline accumulation increased with increasing $ZnSO_4$ concentrations in the current study, Parlak and Yilmaz 2012 said that proline content increased under Zn in three tested plants, Lemna gibba , Lemna minor, and Spirodela polyrrhiza L. Same results were in agreement with Al Khateeb and Al-Qwsemeh 2014 who reported that proline content of both S. nigrum and S. lycopersicum increased with high concentrations of Zn as a result of stress. Proline prevents membrane damage and had a protective role in lipid proxidation induced by metals (Thounaojam et al., 2012). On the other hand current results declared that, the subculture interval every 6 weeks are the better than 4 weeks and these results agree with (Haris and Mantell 1991) and (Grant and Hammatt 1999).

Copper is a micronutrient important for normal plant growth and development. It takes part in processes of photosynthesis, respiration, transport and other physiological and biochemical functions (Yruela 2005). Our results from Tables 5, 6, 7 and 8 indicated that, addition different concentrations of $CuSO_4$ in the MS medium exhibited good growth and healthy of number of shoots, number of embryos, shoots length (cm) and growth vigor/ cluster explant of two types of subculture intervals (4 and 6 weeks) compared with control medium except for the higher concentration of $CuSO_4$ (100.0 Mµ) exhibited weak shoots and symptoms of toxicity. In accordance to an earlier report by Joshi and Kothari 2007 on *Capsicum annuum*, the increasing of Cu concentration in the medium significantly favored the induction of shoot-buds and their elongation in the second stage subculture. Similar findings were also reported by *Elucine coracana* (Kothari et al., 2004) *Stevia ebaudiana* (Jain et al., 2009), *Withania somnifera* (Fatima et al., 2011), *Phoenix dactylifera* (Madi and Al Mayahi 2014) and *Capsicum annuum* (Grozeva 2015). Also Shahied et al., 2015 tested effect different concentrations of copper sulphate (in increasing order) for nodal explant culture of *Rauvolfia tetraphylla*. The cultures maintained on different concentrations of copper sulphate exhibited better growth and multiplication in comparison to control after 4 weeks of culture.

Cu is an important constituent of several enzymes like cytochrome oxidase, ascorbic oxidase, phenolase, diamine oxidase, super-oxide dismutase, as well as plastocyanin, a pigment participating in electron transfer. The optimum Cu concentration in medium positively affects development of membrane system of chloroplasts and Chl content. In our experiments the Chl *a*, Chl *b*, total Chl and car content in the multiplication in vitro of date palm cv. Yellow Maktoum increased with the increasing copper concentrations up to the level 50.0 µM $CuSO_4$, which is in accordance with earlier reports such *Lupinus luteus* (Mourato et al., 2009), *Jatropha curcas* (Khurana-Kaul et al., 2010) and *Rauvolfia serpentine* (Ahmed et al., 2015). Higher concentrations of $CuSO_4$ caused a decline in the photosynthetic pigments this result agree with (Romeo-Puertas et al., 2004 and Vuksanović et al., 2017).

The stimulating effect of Cu can be ascribed to its role in several metabolic activities like protein and carbohydrate metabolism. The various Cu containing enzymes involved in electron transport, protein and carbohydrate biosynthesis might play a role in plant regeneration. From our results in Fig 7, the low concentrations of $CuSO_4$ (10.0 and 25.0 µM) encouraged increasing significant of total protein content compared with the higher concentrations (50.0, 75.0, 100.0 µM) these results were supported by Kothari et al., 2008 and Shahied et al., 2015.

In the present study, proline accumulation increased by increasing $CuSO_4$ concentrations in MS medium and the maximum proline content of 0.78 mg g^{-1} FW was on the MS medium containing 100.0 µM $CuSO_4$ (Fig. 8). These results agree with Fatima et al., 2011 of *Withania somnifera* and Al-Khateeb and Al-Qwasemeh 2014 of Solanum nigrum. The production of proline at higher Cu concentrations correlated with a lower regeneration frequency and a decrease in biomass and pigment content. Such toxic responses of Cu have recently been reported (Jain et a*l*., 2009 and Ahmed 2015). On the other said current results indicated that, the subculture interval every 6 weeks are the better than 4 weeks for all parameters and these results agree with (Haris and Mantell 1991) and (Grant and Hammatt 1999)**.**

Furthermore, zn influences cell division and cell expansion besides having a role in chlorophyll formation and our study in Table 9 showed that during rooting stage, the addition of $ZnSO_4$ with different concentrations from 10.0 to 100.0 µM to rooting medium was beneficial for good growth of shoots and roots compared to the control treatment (having normal zinc concentration in MS basal medium). Additional supply of zinc in rooting medium stimulated rooting percentage and root length (Ali et al., 2000). Tsui 1948 reported that, zinc is required directly for the synthesis of tryptophane and indirectly for the synthesis of auxins.

Also, our results in Table 10 showed that $CuSO_4$ at 75.0 µM was the best concentrations of all growth parameters during rooting stage in vitro (shoots length, number of roots, roots length and growth vigor/explan). These results were line with Jiang et al., 2000 of *Zea mays* and Joshi and Kothari 2007 of *Capsicum annuum* who noticed that 10^{-5} M Cu stimulated root growth. The various Cu containing enzymes involved in electron transport, protein and carbohydrate biosynthesis might play a role in plant regeneration (Purnhauser and Gyulai 1993) that might explain our good results during the rooting stage.

In conclusion, the addition of $ZnSO_4$ and $CuSO_4$ in their different concentrations to the growth medium of date palm cv. Yellow Maktoum during the multiplication stage and also the rooting stage had the greatest effect on the improvement of growth and strong plantlets were able to transfer to the adaptation stage and achieve the highest success rate in agriculture within the acclimatization greenhouse.

REFERENCES

Abahmane, L. 2017. Cultivar-Dependent Direct Organogenesis of Date Palm from Shoot Tip Explants. p. 3-16. In: J.M. Al-Khayri S.M. Jain and V.J. Dinnes (eds.), Date palm protocols. Springer, Berlin.

Ahmad, N. ALatar, A.A. Faisal, M.M. Khan, I. Fatima, N. Anis, M. and Hegazy, A.K. 2015. Effect of copper and zinc on the *in vitro* regeneration of *Rauvolfia serpentine*. Biol Plant. 59: 11-17

Ali, G. Srivastava, P.S. and Iqbal, M. 1999. Morphogenic and biochemical responses of Bacopa monniera cultures to zinc toxicity. Plant Sci. 143:187–193.

Ali, G. Srivastava, P.S. and Iqbal, M. 2000. Influence of Cadmium and Zinc on Growth and Photosynthesis of Bacopa monniera Cultivated *in vitro*. Biol. Plant. 43:599–601.

Al-Khayri, J.M. 2007. Date palm *Phoenix dactylifera* L. micropropagation. p 509–526. In: S.M. Jain and H. Haggman (eds.), Protocols for micropropagation of woody trees and fruits. Springer, Berlin.

Al Khateeb, W. and Al-Qwasemeh, H. 2014. Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of *Solanum nigrum* L., a cropwild relative for tomato; comparative study. Physiol Mol Biol Plants 20:31–39.

Aron, D. 1994. Copper Enzymes In Isolated Chloroplasts. Polyphenoloxidase In *Beta Vulgaris*. Plant Physiol. 24: 1-15.

Bardar, S. Khurana-Kaul, V. Kachhwaha, S. and Kothari, S.L. 2014. Nutrient optimization for improved *in vitro* plant regeneration in *Eclipta alba* (L.) Hassk. and assessment of genetic fidelity using RAPD analysis. Plant Tissue. Cult. & Biotech., 24: 223–234.

Bates, L.S. Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studied. Plant and Soil, 39: 205-207

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of micrograms quantities of protein utilization the principle of protein – dye binding. Analytical Biochemistry 72: 248-254

Brennan, R..F. 2005. Zinc Application and Its Availability to Plants. Ph. D. dissertation, School of Environmental Science, Division of Science and Engineering, Murdoch University.

Broadley, M.R. White, P.J. Hammond, J.P. Zelko, I. and Lux, A. 2007. Zinc in plants. New Phytologist, 173: 677-702.

El Dawayati, M.M Zayed, Z.E. and Sidky, R.A. 2014. An Efficient protocol for the in vitro multiplication of date palm (*Phoenix dactylifra* L.) cv. Gondela to optimize shooting stage. Egypt. J. of Appl. Sic. 29: 318-332.

EL- Jassani, I. F. 2013. Studies on micro propagation of Eucalyptus sp. and *Phoenix dactylifra* L. M. Sc. Thesis, Department of Ornamental, Faculty of Agriculture, Cairo University, Egypt**.**

Fatima, N. Ahmad, N. and Anis, M. 2011. Enhanced in vitro regeneration and change in photosynthetic pigments, biomass and proline content in *Withania somnifera* L. (Dunal) induced by copper and zinc ions. Plant Physiol Biochem. 49:1465–1471.

Fki, L. Masmoudi, R. Kriaa, W. Mahjoup, A. Sghaier, B. Masid, R. Mliki, A. Rival A. and Drira, N. 2011. Date palm micropropagation via somatic embryogenesis. p.47-68. In: S. Jain ; J. Al-Khairy and D. Johonson (eds.), .p . Date Palm Biotechnology .Springer, Dordrecht.

Grant, N.J. and Hammatt, N. 1999. Increased root and shoot production during micropropagation of cherry and apple rootstocks: effect of subculture frequency *Tree Physiology*, 19: 899–903.

Grozeva, S. 2015. Effect of copper levels in the culture medium on shoot regeneration in pepper. Banat's J. of Biotech. 2: 86-91.

Hänsch, R. and Mendel, R.R. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Curr. Opin. Plant Biol. 12:259–266.

Jain, P.K achhwaha, S. and Kothari, S.L. 2009. Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* (Bert.) Bertoni by using high copper levels in the culture medium. Sci. Hort. 119: 315-319

Jiang, W. Liu, D. and Liu, X. 2000. Effect of copper on root growth, cell division, and nucleolus of *Zea mays*. Biol. Plant. 44: 105-109.

Joshi, A. and Kothari, S.L. 2007. High copper levels in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of Capsicum annuum L. Plant Cell Tiss Organ Cult 88:127–133.

Khudsar, T. Mahmooduzzafar Iqbal, M. and Sairam, R.K. 2004. Zinc induced changes in morpho-physiological and biochemical parameters in *Artemisia annua.* - Biol. Plant. 48: 255-260

Khurana-Kaul, V. Kachhwaha, S. and Kothari, S.L. 2010 Direct shoot regeneration from leaf explants of *Jatropha curcas* in response to thidiazuron and high copper contents in the medium Biol. Plant. 54:369–372.

Kothari, S.L. Agarwal, K. and Kumar, S. 2004. Inorganic nutrient manipulation for highly improved in vitro plant regeneration in finger millet-*Eleusine coracana* (L.) Gaertn. In Vitro Cell Dev Biol-Plant 40:515–519

Kothari, C.A. Sharma, M. Kachhwaha, S.and Kothari, S.L. 2008. Micronutrient optimization results into highly improved in vitro plant regeneration in kodo (*Paspalum scrobiculatum* L.) and finger (*Eleusine coracana* (L.) Gaertn.) millets. Plant Cell Tiss and Organ Cult. 94:105-112.

Madi, A. and AL-Mayahi, W. 2014. Effect of copper sulphate and cobalt chloride on growth of the *in vitro* culture tissues for date palm (*phoenix dactylifera* l.) cv. Ashgar. Amer. J. of Agric. and Biol. Sci. 9: 6-18

Mourato, M.P. Martins, L.L.and Campos-Andrada, M.P. 2009. Physiological responses of *Lupinus luteus* to different copper concentrations. Biol. Plant. 53: 105-111.

Mujib, A.S. Banrjee, P. and Ghosh, D.2005. Origin, development and structure of somatic embryos in selected Bulbous ornamentals: BAP as inducer, p 15-24. In: A. Mujib and J. Samaj (eds.), Somatic Embryogenesis, Springer-Verlag, Berlin, Heidelberg.

Murashige, T. and Skoog, F.A. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.

Narula, A. Kumar, S. and Srivastava, P.S. 2005. Abiotic metal stress enhances diosgenin yield in *Dioscorea bulbifera* L. cultures. Plant Cell Rep. 24:250–254

Parlak, K.U. and Yilmaz, D.D. 2012. Response of antioxidant defences to Zn stress in three duckweed species. Ecotoxicol Environ Saf. 85:52–58

Pottino, B.G. 1981. Methods in plant tissue culture. Dept. of Hot. Agric, College. Maryland University. p8–29.

Purnhauser, L. and Gyulai, G. 1993 Effect of copper on shoot and root regeneration in wheat, triticale, rape and tobacco tissue cultures. Plant Cell Tiss Org Cult 35:131–139.

Ramage, C.M. and Williams, R.R. 2002. Mineral Nutrition and Plant Morphogenesis. *In Vitro* Cellu. & Develop. Biol. Plant 38:116-124.

Romeo-Puertas, M.C. Rodriguez-Serrano, M. Corpas, F.J. Gomez, M. Del Rio, L.A. and Sandalio, L.M. 2004. Cadmium induced subcellular accumulation of O_2 and H_2O_2 in pea leaves- Plant Cell Environ. 27: 1122-1134

Rosalind, A. Harris and Mantell, S.H. 2015. Effects of Stage II subculture durations on the multiplication rate and rooting capacity of micropropagated shoots of tree paeony (*Paeonia suffruticosa* Andr.), J. of Horti Sci, 66:95-102.

Shahid, A. Ahmad, N. Anis, M. Alatar, A. and Faisal, A. 2015. Morphogenic responses of Rauvolfia tetraphylla L. cultures to Cu, Zn and Cd ions. Rend. Fis. Acc. Lincei. DOI 10.1007/s12210-015-0491-5.

Sharma, S.S. and Dietz, K.J. 2009**.** The relationship between metal toxicity and cellular redox imbalance. Trends in Plant Sci. 14: 43-50.

Sharma, S. Sharma, P. Datta, S.P. and Gupta, V. 2010. Morphological and Biochemical Response of *Cicer arietinum* L. var. pusa-256 towards an Excess of Zinc Concentration. Life Sci. J. 7:1-110

Snedecor, G.W. and Cochran, W.G. 1972 Statistical Method 6^{th}. The Iowa State University Press, Ames., Iowa U.S.A., p59.

Thounaojam, T.C. Panda, P. Mazumdar, P. Kumar, D. Sharma, G.D. Sahoo, L. and Panda, S.K. 2012. Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Biochem 53:33–39

Tsonev, T. Lidon, F.J.C. 2012. Zinc in plants - An overview. Emir. J. Food Agric. 24: 322-333

Tsui, C. 1948. The Role of Zinc in Auxin Synthesis in the Tomato Plan Americ. J. of Bot., 35: 172-179.

Vuksanović, V. Kovačević, B. Katanić, M. Orlović, S. and Miladinović, D. 2017. *In vitro* Evaluation of Copper Tolerance and Accumulation in *Populus nigra* Arch Biol Sci. 69:679-687.

Yruela, I. 2005.Toxic metal in plants. Copper in plants. Brazil. J. of Plant Physiol. 17:145–156.

Zayed, Z.E. 2014. Effect of different types of cytokinins on the regeneration ability of direct somatic embryos and adventitious shoots induced from immature inflorescences of date palm. Egypt. J. of Appl. Sci., 29: 142-153.

Zayed, Z.E. 2017. Enhanced Indirect Somatic Embryogenesis from Shoot-Tip Explants of Date Palm by Gradual Reductions of 2,4-D Concentration. P 77-88. In: J.M. Al-Khayri, S.M. Jain and VJ.. Dinnes (eds.), Date Palm Biotechnology protocols. Springer, Berlin.

Tables

Table 1. Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on shoots number of date palm cv. Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	14.75	18.25	16.5
10	20.75	26.50	23.62
25	25.50	32.25	28.87
50	32.25	40.00	36.12
75	28.00	34.50	31.25
100	24.57	28.75	26.66
Mean(B)	24.30	25.70	
$LSD_{0.05}$	(A)3.82	(B)1.04	(AB)2.54

Table 2. Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on Secondary embryos number of cluster explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM (A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	5.25	7.32	6.28
10	6.75	7.75	7.25
25	8.25	9.25	8.75
50	10.25	11.75	11.00
75	8.75	8.00	8.37
100	3.20	4.25	3.72
Mean(B)	7.07	8.05	
$LSD_{0.05}$	(A)2.41	(B)1.23	(AB)1.56

Table 3. Effect of different concentrations$ZnSO_4$ and the subculture interval (4 and 6 weeks) on shoots length (cm) of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM (A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	4.25	5.00	4.62
10	4.37	5.37	4.87
25	6.12	5.50	5.81
50	6.25	7.50	6.87
75	6.00	6.25	6.12
100	5.00	5.25	5.12
Mean(B)	5.33	5.81	
$LSD_{0.05}$	(A)0.82	(B)0.35	(AB)0.78

Table 4. Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on growth vigor of clusters explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM (A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	2.50	3.00	2.75
10	2.50	3.50	3.0
25	2.75	3.75	3.25
50	3.75	4.00	3.87
75	3.50	3.75	3.62
100	3.00	2.50	2.75
Mean(B)	3.00	3.41	
$LSD_{0.05}$	(A)0.25	(B)0.23	(AB)0.45

Table 5. Effect of different concentrations $CuSO_4$ and the subculture interval (4 and 6 weeks) on shoots number of date palm cv. Yellow Maktoum after three subculture of culture during multiplication stage.

$CuSO_4$µM (A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	14.75	18.25	16.5
10	26.25	22.00	24.12
25	34.25	38.00	36.12
50	29.00	40.50	34.75
75	22.25	34.75	28.50
100	18.25	28.00	23.12
Mean(B)	24.12	30.25	
$LSD_{0.05}$	(A)4.25	(B)4.02	(AB)3.45

Table 6. Effect of different concentrations$CuSO_4$ and the subculture interval (4 and 6 weeks) on Secondary embryos number of cluster explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$CuSO_4$µM(A)	Subculture interval(B)		Mean(A)
	4weeks	6weeks	
0(Control)	5.25	7.32	6.28
10	6.25	12.50	9.37
25	7.00	11.25	9.12
50	6.20	10.50	8.35
75	5.25	9.00	7.12
100	4.75	6.75	5.76
Mean(B)	5.78	9.55	
$LSD_{0.05}$	(A)1.06	(B)1.89	(AB)1.37

Table 7. Effect of different concentrations $CuSO_4$ and the subcultures interval (4 and 6 weeks) on shoots length (cm) of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$CuSO_4$µM (A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	4.25	5.00	4.62
10	5.50	5.75	5.62
25	5.75	6.50	6.12
50	6.45	7.50	6.97
75	5.00	6.25	5.62
100	3.75	4.25	4.00
Mean(B)	5.11	5.87	
$LSD_{0.05}$	(A)0.44	(B)0.33	(AB)0.73

Table 8. Effect of different concentrations $CuSO_4$ and the subculture interval (4 and 6 weeks) on growth vigor of clusters explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$CuSO_4$µM (A)	Subculture interval(B)		Mean(A)
	4weeks	6weeks	
0(Control)	2.50	3.00	2.75
10	2.75	3.75	3.25
25	3.75	4.00	3.87
50	3.75	4.00	3.87
75	3.25	3.50	3.37
100	2.25	2.50	2.37
Mean(B)	3.04	3.45	
$LSD_{0.05}$	(A)0.43	(B)0.22	(AB)0.47

Table 9. Effect of different concentrations zinc sulphate ($ZnSO_4$) on roots formation of date palm cv. Yellow Maktoum after three subculture from culturing

$ZnSO_4$ µM	Shoot length(cm)	No.of root	Root length(cm)	Growth vigor
Control	12.4	3.4	4.7	2.4
10	15.7	7.2	6.0	2.8
25	15.7	7.2	7.0	3.2
50	16.2	7.6	7.5	3.6
75	16.0	8.2	7.5	3.8
100	16.5	8.1	8.0	4.0
$LSD_{0.05}$	0.47	0.33	0.36	0.32

Table 10. Effect of different concentrations copper sulphate ($CuSO_4$) on roots formation of date palm cv. Yellow Maktoum after three subculture

$CuSO_4$µM	Shoot length(cm)	No.of root	Root length(cm)	Growth vigor
Control	12.4	3.4	4.7	2.4
10	13.7	4.0	6.3	3.2
25	14.4	6.6	8.3	3.8
50	16.9	7.2	8.6	3.8
75	17.6	9.2	7.6	4.0
100	15.4	8.8	6.5	4.0
$LSD_{0.05}$	0.89	0.52	0.45	0.25

Figures

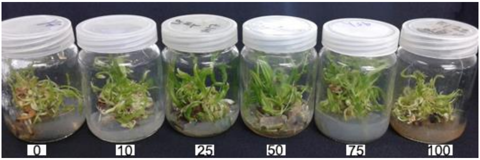

Fig.1. Effect of different concentrations of $ZnSO_4$ on shoot growth

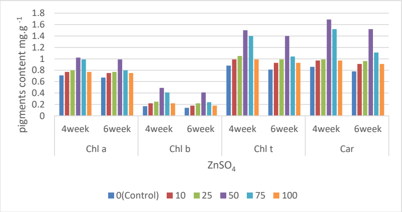

Fig. 2. Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on the pigments content (Chl a, Chl b, Chl t and Car mg.g^{-1} FW) of date palm cv. Yellow Maktoum

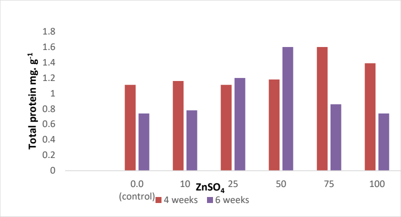

Fig. 3. Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on total protein content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum

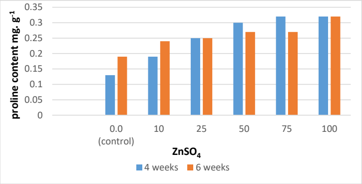

Fig. 4. Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on proline content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum

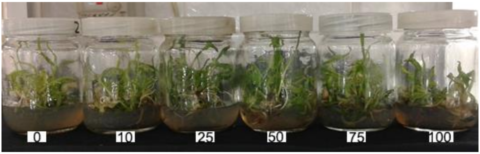

Fig. 5. Effect of different concentrations $CuSO_4$ on shoot growth

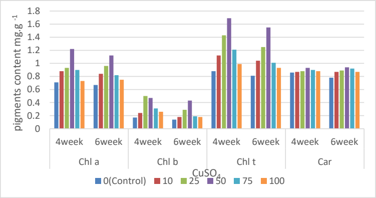

Fig. 6. Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on the pigments content (Chl a, Chl b, Chl t and Car mg.g^{-1} FW) of date palm cv. Yellow Maktoum

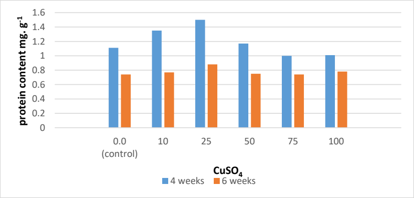

Fig. 7. Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on protein content (mg.g^{-1} FW) of date palm cv. Yellow

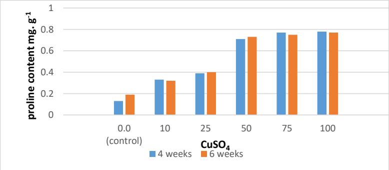

Fig. 8. Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on proline content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum

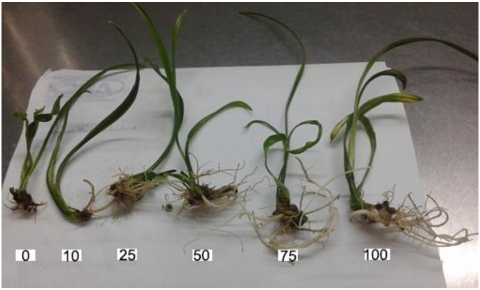

Fig. 9. Effect of different concentration of $ZnSO_4$ on root growth

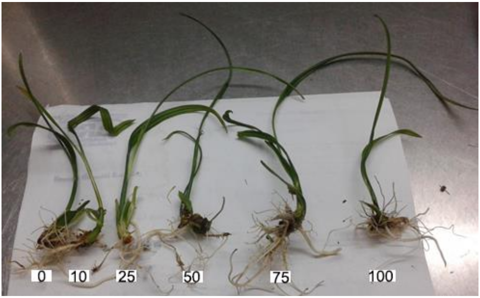

Fig.10. Effect of different concentration $CuSO_4$ on root growth.

Cryopreservation of embryogenic cultures of date palm using encapsulation-dehydration technique and assessment of genetic stability

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Abstract

This study aimed to recognize a method for cryopreservation of two Egyptian date palm cultivars i.e., Bartamoda and Sakkoty using encapsulation-dehydration technique. Embryogenic cultures were proliferated on MS medium supplemented with 10 mg/ l 2, 4-D + 3 mg/l 2iP. The cultures were maintained on medium contained high sucrose concentrations. Encapsulation was performed by suspending the cultures with three sodium alginate followed by immersion in calcium chloride. The beads containing cultures were dipped in liquid nitrogen for using cryotubes. Generally combination of encapsulation and pretreatment with sucrose increased the survival and the recovery percentages for both cultivars. The highest survival percentage was recorded with adding 0.5 M sucrose or 0.7 M sucrose in the pretreatment medium and encapsulated with 4, 5 % sodium alginate. Also, exposing the cultures to desiccation in the air laminar flow for two hours before cryoprservation increased the survival percentage and subsequently the recovery percentages. It was found that Sakkoty cultivar gave higher survival and subsequently the recovery percentages than Bartamoda cultivar. Random Amplified Polymorphic DNA (RAPD) technique was carried out to investigate the genetic stability of cryopreserved tissue cultures of the two date palm cultivars. According to RAPD analysis, plantlets derived from cryopreserved cultures were similar to that derived from non cryoprserved cultures and also with the mother plants.

Keywords: Date palm, embryogenic cultures, cryopreservation, encapsulation, RAPD analysis.

1. 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 the Middle East. There are >2,000 date varieties with differences in color, flavor, shape, size and ripening time (Al-Farsi and Lee, 2008). Countries which hold significant amounts of genetic diversity of date palm have a great responsibility to conserve and safe guard date palm germplasm to utilize for genetic improvement and development of crop cultivars for domestic and foreign markets. Since date palm is a dioecious and heterozygous fruit tree, and for commercial purposes most often vegetatively propagated through offshoots, its germplasm cannot be stored or handled easily by conventional means. At the present, the most common method used to preserve the genetic resources of date palm is as whole plants on- farm (Bettencourt *et al*., 1992). There are, however, several problems with the field genebank. The collections are exposed to natural disasters and attacks by pests and pathogens. Moreover, labor cost and the requirements for technical personal are very high. In addition, distribution and exchange from field genebank is difficult because of the vegetative nature of the material and the greater risk of disease transfer.

Plant tissue culture in combination with molecular biology techniques are of great interest for collecting, characterization, multiplication and storage of date palm germplasm. Miniaturization of explants allows reduction in space requirements and consequently labor cost for the maintenance of germplasm collections (Bekheet, 2011). In this respect, different *in vitro* techniques to preserve date palm cultures have been recognized (Mater, 1987; MyCock *et al*. 1997; Bekheet *et al*., 2001, 2007). One of the principle long-term *in vitro* conservation methods is cryostorage (storage between -79 and -196 ˚C). The major advantage of plant materials at such temperature is that both metabolic process and biological deterioation are considerably slowed or even halted (Engelmann, 1997). In this context, successful cryopreservation requires the optimization of numerous variables including the size of specimen, the correct type and concentration of cryoprotectant, sample water content and rate of freezing and thawing. Otherwise, the capacity to survive storage in liquid nitrogen is dependent upon many factors including genotype, physiological status and pre- and post- freezing manipulations (Ashmore, 1997). The most commonly employed cryoprotective substances are dimethylsulfoxide (DMSO), mannitol, sorbitol, sucrose and polytehyleneglycol (PEG). Encapsulation- dehydration cryopreservation methods are based on a successive osmotic and evaporative dehydration of plant cells (Swan *et al*., 1999). Dehydration techniques allow more flexibility when handling large sample numbers because
the processing is less time-critical than with vitrification (Sakai *et al*., 2000). Encapsulation-dehydration also avoids the use of harmful cryoprotectants as compared to other methods (Moges *et al*., 2004; Shibli *et al*., 1999).

The stored tissue cultures could be considered a potential risk for the regeneration of genetic instability (Brar and Jain 1998). However, molecular markers are used to study the genetic stability of *in vitro* preserved derived plants. DNA-based techniques have the potential to identify polymorphisms represented by differences in DNA sequences. In this respect, RAPD technique was used by several researchers to examine genetic variability in date palm and it has been found to be very efficient and reliable (Saker *et al*., 2000; Ali *et al*. ,2007; Othmani *et al*., 2010). Furthermore, RAPD technique has been used to study the genetic stability of cryopreserved tissue cultures of date palm (Bekheet *et al*., 2007). This study aims to develop a method for *in vitro* cryopreservation of two Egyptian date palm cultivars i.e., Bartamoda and Sakkoty using encapsulation- dehydration technique and investigate the genetic stability of the cryopreserved cultures using RAPD analysis.

2. MATERIALS AND mETHODS

2.1. Plant materials and sterilization

Offshoots about 10- 15 kg of date palm cvs. Bartamoda and Sakkoty were detached from the adult females grown in Aswan governorate and used as plant materials. The outer leaves were removed with the hard bottom and fibrous sheaths belled off. Then the internal leaves were gradually removed and shoot apices (5 cm) in length were taken and kept in an antioxidant solution (100 mg/l ascorbic acid + 150 mg/l citric acid) to stop phenolic compounds formation. For disinfection, shoot tips were immersed in 70 % ethanol for 1 min followed by 50% of sodium hypochlorite solution for 30 min and then explants were rinsed three times by sterile distilled water.

2.2. Culture media and explanting

For establishment of date palm vitro culture, Murashige and Skoog (1962) (MS) medium was solidified by 7 g agar, the pH was adjusted to 5.8 using 0.1 N of either KOH or HCl and autoclaved at 121 ºC and at pressure of 1.2 kg/cm^2 for 20 min. Shoot tips were trimmed to about 1cm in length and were excised with part of sub-meristematic tissues and aseptically cultured on MS medium (supplemented with 2 mg/l dimethyl amino- purine (2iP), 1 mg/l naphthalene acetic acid (NAA), 30g/l sucrose and 3g/l activated charcoal.

2.3. Incubation and induction of embryonic cultures

Cultures were then incubated in a growth chamber at 25 ±2 ºC under darkness conditions and recultured on the same fresh medium for three times (six weeks intervals). Cultures were transferred to MS medium supplemented with 10 mg/l 2,4- dichlorophenoxy acetic acid (2,4-D) and 3 mg/l 2iP. Embryogenic cultures were proliferated and maintained on MS medium supplemented with 2.5 mg/l kinetin (kin) and 0.5 mg/l 2,4-D (El-Ashry *et al*., 2013).

2.4. Cryopreservation encapsulation-dehydration procedures

2. 4. 1. Preparation of sodium alginate solution

Three concentrations i.e., 3, 4 and 5 % (w/v) of sodium alginate were dissolved in distilled water and then sterilized in autoclave for 20 min at 121°C and at a pressure of 1.2 Kg /cm^{-2} and then they were allowed to cool at room temperature 24 ± 2 °C for one hour.

2. 4.2. Preparation of calcium chloride solution

Calcium chloride at 100 mM was prepared by dissolving 14.70 g of $CaCl_2.2H_2O$ in 1000 ml distilled water and then sterilized by autoclave as mentioned before and then were allowed to cool for at least one hour.

2. 4.3. Cultures dehydration

Embryogenic cultures were maintained on medium MS + 2.5 mg/l kin + 0.5 mg/l 2,4-D contained different sucrose concentrations i.e., 0.3,0.5 and 0.7 M . The treatments were incubated at 24°C ±2 in complete darkness for two weeks. At the end of incubation period, each treatment was transferred from the pre-culture medium and placed into Petri dish and then directed to renew sterile air in the laminar air flow for two hours.

2. 4.4. Encpsulation

Encapsulation was performed by suspending the embryogenic cultures with sterilized sodium matrix at the concentrations of 3, 4 or 5 % (w/v) in order to allow capsules formation. Alginate- covered embryogenic cultures of each concentration were dropped individually in Calcium chloride for 30 min on a rotary shaker prior to retrieval and rinsed with sterilized distilled water to remove residues.

2. 4.5. Cryo-storage

Cultures were placed in cryotubes (2 ml) and then plunged into liquid nitrogen (at – 196 °C) container for 48 hour. After the cryopreservation period, cryotubes with explants were placed in – 80 °C for one week. Then cryotubes were placed in water bath at 40 ± 2 °C for rapidly thawing.

2. 5. Survival and recovery

The cryopreserved cultures of each pretreatment were re-cultured on recovery medium which contained ¾ MS strength + 1 g/l activated charcoal + 40 g/l sucrose + 0.5 mg/l BA + 0.5 mg/l Kin + 0.1 mg/l NAA for embryos development. The cultures were incubated for three weeks at 24°C± 2 in darkness to evaluate the survival percentage. Recovery percentage was recorded as percentage of cultures resumed growth after three months.

2. 6. Statistical analysis

All experiments were arranged in a completely randomized design. Data were analyzed using two way analysis of variance with interaction and means was separated using Duncun´s multiple range test at 5 % level test (SAS program 1996) according to Snedecor and Cochran (1982).

2.7. Random Amplified Polymorphic DNA (RAPD) analysis

2.7.1. DNA extraction

- Plant samples (200 mg) were ground and homogenized with extraction buffer (300- 400 μl).
- One volume (700 μl) of chloroform – iso amyl alcohol solution (24: 1) was added to the homogenate and carefully mixed.
- After centrifugation (4 min) at speed 14000 rpm in a bench-top centrifuge, the supernatant was taken up and extracted again with chloroform-iso amyl alcohol solution (24: 1).
- Extraction with chloroform-iso-amyl alcohol was repeated until clarification of the supernatant has taken place.
- The DNA was precipitated by 3 volumes of ice cold absolute ethanol, then carefully mixed 2-3 times and centrifuged for 10 min.

2.7.2. Polymerase Chain Reaction (PCR)

Amplification of PCR was performed in 0.1 ml reaction mixture containing 20 ng template DNA, 0.5 unit Taq polymerase (Promega. USA), 200 μM each of dATP, dCTP, dGTP, dTTP. 10 pmole random primers (A6 (5/- CCCTACCGAC-3), A10 (5´-TCGTTCCGC -3´), A11 (5/- TGGCGACCTG -3/), A12 (5/- GAGGCGTCGG -3/) , and A13(5´- CACCTTTCCC-3´) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 44 cycles, using UNO thermalcycler of Biometra (Germany) as follows: one cycle at 92 °C for 2 min then 44 cycle at 92°C for 30 s, 36°C for 30 s and 72°C for 2 min (for denaturation, annealing and extension, respectively). Reaction mixture was finally incubated at 72°C for 10 min and further 10 min at 62°C. The amplification products were analyzed by electrophoresis in 2% agarose in TAE (Tris-acetate EDTA) buffer, stained with ethidium bromide (0.2 μg /mL) and photographed under UV light.

3. RESULTS AND DISCUSSION

3.1. Effect of sodium alginate concentrations and sucrose treatments on survival of cryopreserved cultures

Results of encapsulation with different concentrations of sodium alginate and sucrose pretreatments on survival of cryopreserved embryogenic cultures of Bartamoda and Sakkoty date palm cultivars are presented in Table (1). Data reveal that the highest mean of survival percentage (45 %) was recorded with adding 0.7 M sucrose in the pretreatment medium and encapsulated with 4, 5 % sodium alginate without a significant difference between the other two sodium alginate concentrations. Survival capacity was diminished with the treatment of low concentrations of sucrose. Moreover, all cultures died when they were cryopreserved without sucrose treatment. By comparing the two cultivars it is found that Sakkoty cultivar gave significantly higher survival percentages than Bartamoda cultivar Table (1). The highest survival percentage of Sakkoty cultivar (60 %) was recorded when the embryos were treated with either 0.5 M or 0.7 M sucrose in the pretreatment medium then exposed to air dessication for two hours and encapsulated with 4 % or 5 % sodium alginate without any significant difference between them.

3.2. Effect of sodium alginate concentrations and sucrose treatments on recovery of cryopreserved cultures

Regarding the recovery percentages, it took the same trend as the survival percentages. The highest recovery percentage was registered with the treatments of 0.5 M sucrose and and capsulated with 4, 5 % sodium alginate since it reached (30% and 25 % respectively) without a significant difference between the two sodium alginate percentage (Table 2) and Fig (1). It is found that Sakkoty cultivar gave significantly higher recovery percentage when it was compared with Bartamoda cultivar. Generally, the highest recovery percentage was recorded with Sakkoty embryos on the same treatments it recorded (50 % and 40 % respectively) without a significant difference between the two sodium alginate percentages.

The encapsulation-dehydration technique is based on the artificial seed technology. Encapsulation-dehydration includes encapsulation of plant material in calcium alginate beads, followed by pregrowth treatment in a medium containing high levels of sucrose. The alginate beads are then dehydrated before freezing using either air-drying in a laminar flow hood or by exposure to silica gel (Ashmore, 1997). In the current study, survival and regrowth of encapsulated cryopreserved embryonic cultures of Bartamoda and Sakkoty date palm cultivars tended to increase with increasing the sucrose concentration during dehydration. Significant variations in survival and regrowth rates of cryopreserved cultures were obtained among the different concentrations of dehydration treatments. This might indicate that increased sucrose concentration in the pretreatment medium would lead to accumulation of solutes inside the cells resulting in maintaining the integrity of plasma and inner membranes during dehydration and freezing (Moges *et al*., 2004; Plessis *et al*., 1993). Our results are in line with those obtained by Subaih *et al.* (2007). The mentioned that high survival of the encapsulation dehydration method was optimized for date palm when cryoprserved calli were pretreated with 0.3 sucrose for 2 days followed by 2 h of dehydration. Mycock *et al.* (1997) on their study on cryopreservation of somatic embryos of date palm reported that drying samples down to the range of 0.7-1.2 $g.g^{-1}$ allowed for a 32% survival rate. However, Wang *et al*. (2002) found that a maximum growth of encapsulated shoot tips of ´Troyer' citrange was obtained when the sucrose concentrations ranged from 0.15 M to 0.29 M. In this respect, in their study on cryopreservation of cell cultures of date palm, Al-Bahrany and El Kharyri (2012) mentioned that, the highest colony formation, callus weight and embryo number were associated with cell pretreated with 0.75 M sucrose before cryopreservation.

3.3. Random Amplified Polymorphic DNA (RAPD) analysis

Results for RAPD analysis of *in vitro* encapsulated embryos revealed that the used primers (A10, A12and A13) gave total 15 bands and 2 polymorphic bands with Bartamoda cultivar. Primer A10 produced 7 bands, A12 gave 5 whereas 3 bands were proliferated by A13. The average percentage of polymorphism recorded for the three primers was 11.33 % (Table 3). However data of amplification products of cryopreserved embryonic cultures of Sakkoty cultivar indicted that the three primers used gave an overall total 21 bands with 2 polymorphic bands (one polymorphic band for primer A10 and one polymorphic band for A12). A10 primer produced 8, A12 gave 7 and A13 produced 6 bands (3 bands). The overall percentage of polymorphism recorded for all three primers was 8.83 % (Table 3).

In this study, the fragment patterns visualized after gel electrophoresis of revealed genetic similarity between the cryopreserved and non-cryoprserved embryonic cultures of the two date palm cultivar by comparing their RAPD profiles for each of the three primers. The obtained results confirm the theory of metabolic activities at temperatures of liquid nitrogen (LN) are reduced to zero. In this respect, A large number of reports showing no evidence of morphological, cytological, biochemical, or molecular alterations in plants from storage at -196°C (Harding, 2004). The present results are accordance with Bekheet *et at*. (2007). They mentioned that, plantlets derived from cryopreserved cultures were identical to that derived from non-treated cultures and both were similar with the field grown plants. Srivastava *et al.* (2009) reported that the similarity coefficients was observed 0.932-0.955 with RAPD marker in *Cineraria maritima* plants grown after storage of encapsulated microshoots. Recently, Alansi *et al*., (2017), investigated the genetic fidelity of date palm plantlets vs. Sagai and Khalas' derived from somatic embryogenesis before and after cryopreservation with liquid nitrogen using inter simple sequence repeat (ISSR) technique. The results indicate that plantlets of the two cultivars derived from embryogenic calli after cryopreservation (+LN) and non-cryopreservation showed high similarity (98.0 %) to their mother plants at the genetic level. In this respect, many researchers did not find any changes in morphology, cytology, biochemistry, or molecular markers in plants stored in LN (Ryynänen and Aronen, 2005; Harding, 2004.

CONCLUSION

In this study, we investigate a method for cryopreservation of two Egyptian date palm cultivars i.e., Bartamoda and Sakkoty using encapsulation-dehydration technique. This method is useful for storage large types of explants specially those sensitive to physical damage caused by ultra-low temperature storage. We found pretreatment producers are very important in cryopreservation process of embryogenic cultures of date palm. It has observed that 0.5 M sucrose-preconditioned embryoic cultures can tolerate freeze storage and subsequently gave best survival and recovery. To realize comprehensive cryo-storage of date palm tissue cultures, further development of cryopreservation techniques are required.

REFERENCES

Al-Farsi, M.A. and C.Y. Lee, 2008. Nutritional and functional properties of dates: a review. Crit. Rev. Food Sci. Nutr. 48: 877-887.

Al-Bahrany, A.M. and Al-Khayri, J.M. (2012). Optimizing in vitro cryopreservation of date palm (Phoenix dactylifera L.). Biotechnology, 11(2): 59-66.

Ali T.A., Jubrail J.M., Jassim A.M. 2007. The use of RAPDs technique for the detection of genetic stability of the regenerated plantlets (Barhi cv.) in Iraq. Acta Horticutura. 736: 127-134.

Ashmore, S.E., 1997. Status report on the development and application of in vitro techniques for the conservation of plant genetic resources, International Plant Genetic Resources Institute, Rome, Italy.

Bekheet, S.A., 2011. In vitro conservation of date palm germplasm. In: Jain SM, Al- Khayri JM, Johnson DV (eds). Date Palm Biotechnology, pp: 337-360. Springer, Netherlands.

Bekheet, S.A., H.S. Taha and M.M. Saker, 2001. In vitro long-term storage of date palm. Biol Plant. 45: 121-124.

Bekheet, S.A., H.S. Taha, M.E. Solliman and N.A. Hassan, 2007. Cryopreservation of date palm (Phoenix dactylifera L.) cultured in vitro. Acta Hort., 736: 283-291.

Bettencourt, E., T. Hazekamp and M.C. Perry, 1992. Directory of germplasm collections. 6.1. Tropical and subtropical fruits and tree nuts. IBPGR, Rome.

Brar DS, Jain SM (1998) Somaclonal variation: mechanism and applications in crop improvement. In: Jain SM, Brar DS, Ahloowalia BS (eds.) Somaclonal variation and induced mutations in crop improvement. Kluwer, Dordrecht, pp 15–37.

El-Ashry, A.A., A.D. Shaltout, M.K. El-Bahr, Abd El Hamid, M.A. Matter and S.A. Bekheet (2013). In vitro preservation of embryogenic cultures of two Egyptian dry date palm cultivars at darkness and low temperature conditions. Journal of Applied Sciences Research, 9(3): 1985-1992.

Engelmann, F., 1997. In vitro conservation methods. In: Ford-Lloyd, B.V, Newbury, J.H. and Callow, J.A. (eds). Biotechnology and Plant Genetic Resources: Conservation and Use. CAB International, Wall ingford, UK, pp: 119-162.

Harding, K. (2004). Genetic integrity of cryopreserved plant cells: A review. CryoLetters 25: 3-22.

Mater, A.A., 1987. Production of cryogenic freezing of date palm germplasm and regeneration of plantlets from frozen material. Iraq J Agric Sci Zanco, 5: 35-49.

Moges, A.D.; Karam, N.S.; Shibli, R.A. 2004. Cryopreservation of African violet (Saintapulia ionantha Wendl.) shoot tips. In Vitro Cell. Develop.Biol.-Plant., 40:389-398.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

MyCock, D.J., P. Berjak, N.W. Pammenter and C.W. Vertucci, 1997. Cryopreservation of somatic embryos of Phoenix dactylifera L. In: Ellis RH, Black M, Murdoch AL, Hong TD (eds.), Basic Applied Aspects of Seed Biology. , pp 75–82. Kluwer, Dordrecht.

Othmani, A.; S. Rhouma; C. Bayoudh; R .Mzid; N. Drira and M .Trifi (2010). Regeneration and analysis of genetic stability of plantlets as revealed by RAPD and AFLP markers in date palm (Phoenix dactylifera L.) cv. Deglet Nour. International Research J. Plant Sci. 1(3): 48-55.

Plessis, P.; Leddet, C.; Collas, A.; Dereuddre, J. 1993. Cryopreservation of Vitis vinifera L. cv Chardonnay Shoot Tips by Encapsulation-dehydration: Effects of Pretreatment, Cooling and Post Culture Conditions. Cryo-Letters, 14:309-320.

Ryynänen, L. and T. Aronen. 2005. Genome fidelity during short-and long-term tissue culture and differentially cryostored meristems of silver birch (Betula pendula). Plant Cell, Tissue & Organ Cult., 83(1): 21-32.

Sakai, A.; Matsumoto, T.; Hirai, D.; Niino, T. 2000. Newly developed encapsulation-dehydration protocol for plant cryopreservation. Cryo-Letters, 21:53-62.

Saker M. M.; S. A. Bekheet; H. S. Taha; A. S. Fahmy and H. A. Moursy (2000). Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biologia Plantarum, 43 (3):347-351.

Shibli, R.A.; Smith, M.A.L.; Shatnawi, M.A. 1999. Pigment recovery from encapsulated-dehydrated Vaccinium pahalae (ohelo) cryopreserved cells. Plant Cell, Tiss.Org. Cult., 55: 119-123.

Snedecor, G.W. and W.G. Cochran (1982). Statistical Methods. 7 th Ed. The Iowa State Univ. Press. Ames. Iowa, USA.

Srivastava, V. , S.A. Khan and S. Banerjee. 2009. An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: Cineraria maritima following six months of storage. Plant Cell, Tissue & Organ Cult. (PCTOC)., 99(2): 193-198.

Subaih, W.S., M.A. Shatnawi, and R.A. Shibli, 2007. Cryopreservation of date palm (Phoenix dactylifera) embryogenic callus by encapsulation-dehydration,vitrification and encapsulation-vitrification. Jordan J Agricultural Sciences, 3: 156-171.

Swan, T.W.; O′Hare, D.; Gill, R.A.; Lynch, P.T. 1999. Influence of pre=culture conditions on the post-thaw recovery of suspension cultures of Jerusalem artichoke (Helianthus tuberosus L.). Cryo-Letters., 20: 325-336.

Wang, Q. Gafny, R.; Sahar, N.; Sela, I.; Mawassi, M.; Tanne, E.; Perl, A. 2002.Cryopreservation of Grapevine (Vitis Vinifera L.) Embryogenic Cell Suspension by Encapsulation-dehydration and Subsequent Plant Regeneration. Plant Sci., 162: 551-558.

Tables

Table (1). Effect of sodium alginate, sucrose concentrations and air desiccation on survival of cryopreserved embryonic cultures of two date palm cultivars i.e., Bartamoda and Sakkoty.

Treatments	Survival (%)		
	Bartamoda	**Sakkoty**	**Means**
- Encapsulation with 3% sodium alginate	0.0 c	0.0 c	0.0 D
- Encapsulation with 4% sodium alginate	0.0 c	0.0 c	0.0 D
- Encapsulation with 5% sodium alginate	0 .0 d	0.0 d	0.0 D
- 0.3 M sucrose + 3% sodium alginate +2hr. air desiccation	0.0 d	0.0 d	0.0 D
- 0.3 M sucrose + 4% sodium alginate +2hr. air desiccation	10.0 d	20.0 c	15.0 C
- 0.3 M sucrose + 5% sodium alginate +2hr. air desiccation	20.0 c	30.0 b	25.0 B
- 0.5 M sucrose + 3% sodium alginate +2hr. air desiccation	20.0 c	30.0 b	25.0 B
- 0.5 M sucrose + 4% sodium alginate +2hr. air desiccation	20.0 c	60.0 a	40.0A
- 0.5 M sucrose + 5% sodium alginate +2hr. air desiccation	20.0 c	60.0 a	40.0 A
- 0.7 M sucrose+3% sodium alginate +2hr. air desiccation	20.0 c	30.0 b	25.0 B
- 0.7 M sucrose + 4% sodium alginate +2hr. air desiccation	30.0 b	60.0 a	45.0 A
- 0.7 M sucrose + encapsulation with 5% sodium alginate + 2 hr. air desiccation	30.0 b	60.0 a	45.0 A
- Means	14.16B´	29.1A'	

Means with the same letter (s) are not significantly differed at 5 % level

Table (2): Effect of sodium alginate, sucrose concentrations and air desiccation on recovery of cryopreserved embryonic cultures of two date palm cultivars i.e., Bartamoda and Sakkoty.

Treatments	**Recovery** (%)		
	Bartamoda	**Sakkoty**	**Means**
- Encapsulation with 3% sodium alginate	0.0 d	0.0 d	0.0 C
- Encapsulation with 4% sodium alginate	0.0 d	0.0 d	0.0C
- Encapsulation with 5% sodium alginate	0.0 d	0.0 d	0.0C
- 0.3 M sucrose + 3% sodium alginate +2hr. air desiccation	0.0 d	0.0 d	0.0 C
- 0.3 M sucrose + 4% sodium alginate +2hr. air desiccation	0.0 d	10.0d	5.0 BC
- 0.3 M sucrose + 5% sodium alginate +2hr. air desiccation	0.0 d	10.0 c	5.0 BC
- 0.5 M sucrose + 3% sodium alginate +2hr. air desiccation	0.0 d	20.0 b	10.0 B
- 0.5 M sucrose + 4% sodium alginate +2hr. air desiccation	10.0 c	50.0 a	30.0 A
- 0.5 M sucrose + 5% sodium alginate +2hr. air desiccation	10.0 c	50.0 a	30.0 A
- 0.7M sucrose+3% sodium alginate +2hr. air desiccation	0.0 d	20.0 b	10.0 B
- 0.7M sucrose + 4% sodium alginate +2hr. air desiccation	10.0 c	40.0a	25.0 A
- 0.7M sucrose + encapsulation with 5% sodium alginate + 2 hr. air desiccation	10.0 c	40.0 a	25.0 A
- Means	3.33B´	20.0A'	

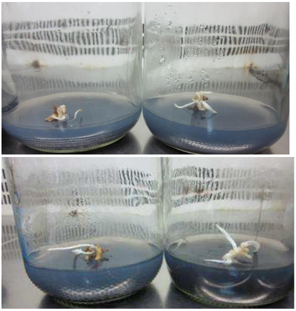
Means with the same letter (s) are not significantly differed at 5 % level

Table (3): Total number of bands, polymorphic bands and percentage of polymorphism as revealed by RAPD markers of cryopreserved embryonic cultures of Bartamoda and Sakkoty cultivars using the three primers.

Cultivars	Primer code	Sequence 5--------3	Total no. of bands	Polymorphic bands	Polymorphism %
Bartamoda	A10	TCGTTCCGC	7	1	14
	A12	GAGGCGTCGG	5	1	20
	A13	CACCTTTCCC	3	0	0
Total			**15**	**2**	**11.33**
Sakkoty	A10	TCGTTCCGC	8	1	12.5
	A12	GAGGCGTCGG	7	1	14
	A13	CACCTTTCCC	6	0	0.00
Total			**21**	**2**	**8.83**

T,C,G and A refer to Thyamine, Cytosine, Guanine and Adenine, respectively

Figures



(A)

(B)

Fig. (1) Re-growth of cryopreserved embryonic cultures of Bartamoda (A) and Sakkoty (B) on ¾ MS strength + 1 g/l activated charcoal + 40 g/l sucrose + 0.5 mg/l BA + 0.5 mg/l Kin + 0.1 mg/l NAA

Effect of light conditions on germination and conversion of indirect date palm somatic embryos to plants

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Abstract

Optimization of date palm somatic embryo multiplication protocols has been the central focus of research, but embryo germination and conversion to plants remains poorly studied in many palm species. In the present study, effect of light or darkness on germination and development of date palm somatic embryos to plants has been studied. The observed results indicated that darkness was associated with increased proliferation and germination of somatic embryos. The darkness significantly stimulated elongation and conversion of somatic embryos to plants. Incubation in darkness was required one month to occur the germination and elongation of somatic embryo, while incubation in light was required four months. Somatic embryo grown in darkness had more protein than other grown in light. Our results are beneficial for the efficient and rapid micropropagation protocol of the date palm.

Key words: Date palm; somatic embryogenesis; light; dark; protein; phenolics

1. INTRODUCTION

Tissue culture comprises several techniques for the mass propagation of selected genotypes. In the case of palms, the most promising and efficient micropropagation technique is somatic embryogenesis. Ever since somatic embryogenesis was first induced in oil palm several decades ago, 19 palm species have been induced to develop somatic embryos from several types of explants. Optimization of somatic embryo multiplication protocols has been the central focus of research, but embryo maturation and conversion remains poorly studied in many palm species. (Ree J.F. and Guerra M.P., 2015).

Somatic embryogenesis is the process by which somatic cells develop into somatic embryos after a series of biochemical and morphological changes (Quiroz-Figueroa *et al.,* 2006), and the formed embryos are morphologically similar to zygotic embryos. (Mazri M.A. and Mezian R., 2015).

Somatic and zygotic embryogenesis have cellular and genetic features in common during both histodifferentiation and the later acquisition of physiological traits associated with maturation.(Patrick von Aderkas *et al.,* 2015).

During maturation, the morphology of the immature globular embryo changes. The cotyledon elongates and specialized tissues develop, including the plumule, the coleoptile housing the plumule, and the radicle. Longitudinal sections of maturing date palm somatic embryos showed differentiated vascular systems along the cotyledon (Bhati and Chandra 2013). Like zygotic embryos, somatic embryos acquire reserves, such as starch (Gomes *et al*., 2014), but they acquire fewer amounts of many essential molecules. Date palm zygotic embryos were found to contain higher levels of ascorbic acid, phenols, flavonoids, and free amino acids (Zein Eldin and Ibrahim, 2015).

Somatic embryo conversion, similar to germination of zygotic embryos, is the transition from an embryo to a plant. Success depends on the formation of both the radicle and plumule during early SE and the accumulation of reserve compounds sufficient to fuel plantlet growth until it can sustain itself through photosynthesis. PGRs have often been shown not to be essential for converting mature somatic embryos, such as in date (Bekheet *et al*., 2001) and macaw palm (Moura *et al*., 2009).

The morphological and physiological consequences of light for somatic embryo development have remained unstudied because, to some extent, light is not a factor in zygotic embryo development. Gymnosperm embryogenesis takes place in the dark interior of closed cones or, in the case of individual ovules such as those of yew, in low light conditions. Light is a factor that is studied post-germination, when the plant becomes autotrophic.

The few studies on light's effect on embryogenesis are confined to angiosperms (Torne *et al*., 2001; Park *et al*., 2010).

Somatic embryos have been shown to have a protein deficit in several palm species, including date (Sané *et al.,*2006; Zein Eldin and Ibrahim 2015), oil (Aberlenc-Bertossi *et al*., 2008,(and macaw palm (Moura *et al*.,2010).

In spite of such powerful effects, abiotic factors are not commonly studied experimentally in vitro. In particular, the effect of light is often overlooked.

In the study presented here we tested the hypothesis that light makes a difference during the germination and conversion of date palm somatic embryos to plants. We compared somatic embryos matured and germinated in light with those matured and germinated in darkness. We also compared somatic embryos converted to plants in light with those converted to plants in darkness.

2. MATERIALS AND METHODS

2.1. Plant material:

Shoot tips of date palm (Phoenix dactylifera L.) cultivar Sultana were disinfected and cultured on callogenesis induction medium containing Murashige and Skoog (MS) salt and vitamins (Murashige and Skoog, 1962), 30 g/l sucrose, 150 mg/l activated charcoal, 7 g/l agar, 5mg/l of BAP and 5mg/l of 2,4-D (Zouine and El Hadrami, 2007; Abohatem *et al*., 2017). For embryogenesis induction, the friable callus formed after 6–8 months culture, was selected and transferred onto medium containing 0.1 mg/l of BAP and 0.5 mg/l of 2,4-D (El Hadrami and Baaziz, 1995; Abohatem *et al.,* 2011). Tissues were incubated at 25±2 ◦C in the dark and subcultured to freshly medium every 5 weeks until the initiation of embryogenic calli.

2.2. Maturation of indirect somatic embryos:

Maturation of somatic embryos are conducted on MS medium diluted a half without plant growth regulator. To test the effect of light on maturation of somatic embryos a set of 0.2 g embryogenic callus masses were placed in either the light or dark for the entire maturation period. Experiments were performed three times. Cotyledons were counted from a minimum of 100 mature embryos per treatment.

2.3. Germination of indirect somatic embryos and conversion into plantlets:

Advanced cotyledonary somatic embryos were picked from the maturation medium after 6 weeks of culture and transferred to germination medium. The germination medium was MS medium supplemented with 0.1 mg NAA and 0.15 g AC.

To test the effect of light on germination of somatic embryos and conversion into plantlets, somatic embryos were placed in either the light or dark for the entire germination of somatic embryos and conversion into plantlets period.

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. 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

3.1. Effect of light on number of mature somatic embryos

There was no significant effect of light on the numbers of embryos that were able to mature. For light treatments, there was an average of 18 somatic embryos per 0.2 g fresh weight compared with 21 in the dark. (Table 1**,** Figure 1)

3.2. Effect of light conditions on germination of indirect somatic embryos and conversion into plantlets:

There was effect of light on the germination of embryos. For light treatments, Frequency of germination (%) was an average of 81 % somatic embryos compared with 92 % in the dark. (Table 1**,** Figure 1).

There was significant effect of light on conversion of date palm somatic embryos to plantlets. The darkness significantly stimulated elongation and conversion of somatic embryos to plantlets. In addition, the darkness significantly decreased period for conversion of somatic embryos to plants from 4 month to one month (Table 1), Incubation in darkness was required one month (Figure 2 B) to occur the germination and elongation of somatic embryo, while incubation in light was required four months. (Figure 2 A).

3.3. Effect of light conditions on proteins content and phenolics of somatic embryos:

There was significant effect of light on proteins content of somatic embryos. At the last stage of somatic embryos conversion (4 week), somatic embryos in the dark treatment had more protein content (112.67 ± 6.3 µg /g FW) than embryos in the light treatment (94.58± 4.6 µg /g FW). (Table 2). At the last stage of somatic embryos conversion (4 week), somatic embryos in the light treatment had higher concentration of phenolics than embryos in the dark treatment.

4. DISCUSSION

Protein accumulation and phenolic compound production are both influenced by light during embryogenesis. Zygotic embryos that develop within megagametophytes in near complete darkness from ovules centrally located in closed cones do not produce phenolic compounds. By comparison, somatic embryos produce phenolic compounds abundantly in light, as well as in the dark. Light also affects protein accumulation, which is greater in dark-grown embryos (zygotic or somatic) than in light-grown somatic embryos. (von Aderkas P. 2015). These findings contribute new information to our understanding of the influence of light during date palm embryogenesis.

There are probably other effects of light to be discovered, because the in vitro effects of light have been relatively little studied. Recent reviews of somatic embryogenesis make no mention of the effect of light.

In date palm, experiments on the effect of light have not been carried out during somatic embryo growth, but only on maturation and formation of somatic embryos. **(**Ree J. F. and Guerra M. P. 2015**).**

Culture in either light or dark has been rarely studied in palm somatic embryos. No significant difference was found for date palm somatic embryos (Bhaskaran and Smith 1992). Somatic embryo conversion, similar to germination of zygotic embryos, is the transition from an embryo to a plant. Success depends on the formation of both the radicle and plumule during early Somatic embryo and the accumulation of reserve compounds sufficient to fuel plantlet growth until it can sustain itself through photosynthesis. PGRs have often been shown not to be essential for converting mature somatic embryos, such as in date (Bekheet *et al.,* 2001). Our results on the effect of light on the differential accumulation of storage products are important because they clearly pinpoint peculiarities unique to somatic embryogenesis. The influence of light can be general or specific within the embryo itself. As we have shown, protein body formation occurs throughout the embryo growth.

The high accumulation of protein in dark-treated embryos in our experiment, which was greater than in light, points to the significance of this organ in providing nutritional storage support for developing somatic embryos to plantlets. Somatic embryos are able to perform as well as zygotic embryos even though somatic embryos lack the surrounding storage productrich megagametophyte with which zygotic embryos are endowed. Although protein accumulation in somatic embryos is generally considered to be under the control of ABA (Roberts, 1991), light also affected protein accumulation in larch somatic embryos matured on ABA.

Zygotic and somatic embryogenesis in palms differs not only in the amount of protein but in the kinds of protein that accumulate (AberlencBertossi *et al*., 2008). Studies comparing zygotic and somatic embryogenesis record so many differences (Jones and Rost, 1989; Alemanno *et al*., 1997; Ka¨rkonen, 2000). The differences in protein content between light- and dark-grown somatic embryos have an effect on germination performance.

The importance of this study lies in a nuanced aspect of somatic embryos growth. Somatic embryos that develop in the dark, as is the case for zygotic embryos inside an ovule, are not exposed to light, which would appear to eliminate light as a factor in development.

This paper offers more support for a new interpretation of the darkness role in nutrition and embryo development. Our results are beneficial for the efficient and rapid micropropagation protocol of the date palm. This method will be significantly decreased period for micropropagation protocol of the date palm.

REFERENCES

Aberlenc-Bertossi F.; Chabrillange N.; Duval Y.; Tregear J. 2008. Contrasting globulin and cysteine proteinase gene expression patterns reveal fundamental developmental differences between zygotic and somatic embryos of oil palm. Tree Physiol 28:1157–1167

Abohatem M.; Zouine, J.; El Hadrami I. 2011.Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. scientia Horticulturae 130 : 344–348.

Abohatem M., Bakil Y., Baaziz M. 2017. Plant Regeneration from Somatic Embryogenic Suspension of date palm. In: Al-Khayryi J., Jain S., Johnson D. (eds) Date palm biotechnology protocols volume 1. Motheds in Molecular Biology, vol 1637. Humana Press, New York, NY.

von Aderkas P. ; Teyssier C. ; Charpentier J.P.; Gutmann M. et al. 2015. Effect of light conditions on anatomical and biochemical aspects of somatic and zygotic embryos of hybrid larch (Larix3marschlinsii). Annals of Botany doi:10.1093/aob/mcu254.

Alemanno L., Berthouly M., Michaux-Ferrie `re N. 1997. A comparison between Theobroma cacao L. zygotic embryogenesis and somatic embry-genesis from floral explants. In vitro Cellular and Developmental Biology– Plant 33: 163–172.

Bekheet S.A.; Saker M.M.; Taha H.S.; Moursy H.A. 2001. Plant regeneration via somatic embryogenesis in date palm (Phoenix dactyliferaL.). Arab J Biotechnol 4:111–118.

Bhaskaran S., Smith R.H. 1992. Somatic embryogenesis from shoot tip and immature inflorescence of Phoenix dactylifera cv. Barhee. Plant Cell Rep 12:22–25.

Bhati A. and Chandra A. 2013. Morphological and anatomical aspects of callogenesis and somatic embryogenesis of date palm (Phoenix dactyliferaL.). Trends Biosc 6:28–30

El Hadrami I. and baaziz M. 1995. somatic embryogenesis and analysis of peroxidase in Phoenix dactylifera L. bio Plant 37: 197-203

Lecouteux, c. G.; Lai, F. M. ; bryan, D. and Mc Kresie, b. D. (1993). Maturation of Alfalfa (Medicago setiva L.) somatic embryos by abscisic acid, sucrose and chilling stress. Plant sci. 94: 207-213.

Jones T.J. and Rost T.L. 1989. The developmental anatomy and ultrastructure of somatic embryos from rice (Oryza sativa L.) scutellum epithelial cells. Botanical Gazette 150: 41–49.

Gomes H.T., Bartos P.M.C., Silva C.O., do Amaral L.I.V., Scherwinski-Pereira J.E. 2014. Comparative biochemical profiling during the stages of acquisition and development of somatic embryogenesis in African oil palm (Elaeis guineensisJacq.). Plant Growth Regul 74:199–208

Ka ¨rkonen A. 2000. Anatomical study of zygotic and somatic embryos of Tilia cordata. Plant Cell Tissue and Organ Culture 61: 205–21

Quiroz-Figueroa F.R., Rojas-Herrera R., Galaz-Avalos R.M., Loyola-Vargas V.M. 2006. Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. Plant Cell Tissue Organ Cult 86: 285-301.

Mazri M.A.and Meziani R.2015. Micropropagation of Date Palm: A Review. Cell Dev Biol 4: 160. doi:10.4172/2168-9296.1000160

Moura E.F., Ventrella M.C., Motoike S.Y. 2010. Anatomy, histochemistry and ultrastructure of seed and somatic embryo of Acrocomia aculeata (Arecaceae). Sci Agric 67:399–407

Moura E.F., Motoike S.Y., Ventrella M.C., de Sá Júnior A.Q., Carvalho M. 2009. Somatic embryogenesis in macaw palm Acrocomia aculeate from zygotic embryos. Sci Hortic 119:447–454.

Murashige T. and Skoog F. 1962. A revised mediumfor rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum 15:473–497.

Ree J. F. and Guerra M. P. 2015. Palm (Arecaceae) somatic embryogenesis. In Vitro Cell.Dev.Biol.—Plant. DOI 10.1007/s11627-015-9722-9

Roberts D.R. 1991. Abscisic-acid andmannitol promote early development, mat-uration and storage protein accumulation in somatic embryos of interior spruce. Physiologia Plantarum 83: 247–254.

Park S-Y., Yeung E.C., Paek K-Y. 2010. Endoreduplication in Phalaenopsis is affected by light quality from light-emitting diodes during somatic embryo- genesis. Plant Biotechnology Reports 4: 303–309.

Sané D., Aberlenc-Bertossi F., Gassama-Dia Y.K., Sagna M., Trouslot M.F. ,Duval Y., Borgel A. 2006. Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of date palm (Phoenix dactylifera). Ann Bot 98:301–308

Torne J.M., Moysset L., Santos M., Simon E. 2001. Effects of light quality on somatic embryogenesis in Araujia sericifera. Physiologia Plantarum 111:405–411.

Zein Eldin A.F.M.and Ibrahim H.A. 2015. Some biochemical changes and activities of antioxidant enzymes in developing date palm somatic and zygotic embryos in vitro. Ann Agric Sci 60:121–130.

Zouine J.; El Hadrami I. 2007. Effect of 2,4-D, glutamine and bAP on embryogenic suspension culture of date palm (Phoenix dactylifera L.). Sci Horticult. 112: 221-226.

Tables

Table 1. Effect of light conditions on production number of somatic embryos, frequency of germination, frequency of conversion of germinated somatic embryos into plantlets and average time taken for conversion embryos to plantlets.

Light condition	Average number of somatic embryos per 200 mg FW of embryogenic callus	Frequency of germination (%) (germinated/embryos tested)	Frequency of conversion (%) (plantlets/germinated embryos tested)	Average time taken for conversion embryos to plantlets (week)
16/8 h light/dark photoperiod	18	81	92	16
Darkness	21	92	96	4

Table 2: Effect of light conditions on proteins content and phenolics of somatic embryos.

Light condition	**Total protein µg / g FW**	**Phenols mg / g FW**
16/8 h light/dark photoperiod	94.58 ± 4.6	0.42 ±0.08
darkness	112.67 ± 6.3	0.38 ±0.06

Figures

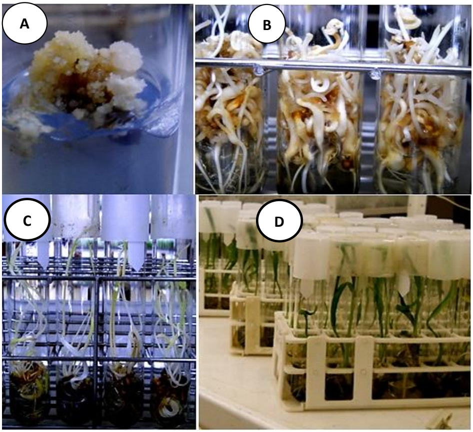

Figure 1. Effect of light conditions on germination of somatic embryos and conversion into plantlets. A. Embyrogenic callus. B. Matured somatic embryos in dark. C. Converted somatic embryos to plantlets in dark. D. Plantlet obtained from a converted somatic embryo.

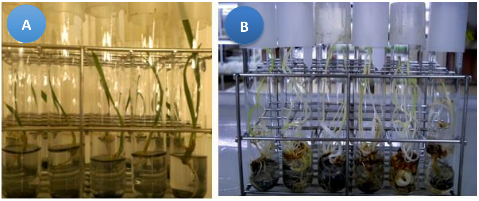

Figure 2. Effect of light conditions on time taken for conversion embryos to plantlets. A. Development stages of somatic embryos in light. B. Converted somatic embryos to plantlets in dark.

A new interspecific date palm hybrid

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Abstract

Crop improvement research in date palm (*Phoenix dactylifera* L.) lags behind due to its slow growth and long life cycle. High quality fruit production in date palms depends on crop management practices such as leaves pruning, removal of leaf spines, pollen dusting, bunch thinning, fruit bagging and harvest. These operations are easy when the palm trees are up to 3 m in height and very difficult when the trees grow taller. Climbing on tall palm trees for these operations is difficult and expensive nowadays. Therefore, we undertook a research activity in our laboratory to develop short date palm trees through interspecific hybridization. The tissue culture date palm orchard maintained at the Kuwait Institute for scientific research (KISR) was used for the experimentation Date palm cultivars Barhi, Madjhool and Sultana were used as female parent and *Phoenix pusilla* was used as male parent. Selected female date palm inflorescences were dusted with *p. pusilla* pollen carefully and bagged immediately after the pollen dusting to avoid pollen mixing. Normal fruit development, growth and ripening was occurred similar to the bunches pollinated with date palm pollen. However, the seed development was arrested and the embryos aborted at the ripening stage due to the failure in endosperm development during seed formation. Therefore, interspecific hybrid embryos were isolated from the immature fruits and germinated *in vitro*. Rooted hybrid plantlets were produced, acclimatized and planted in the field. The first interspecific date palm hybrid was planted in the field during 2009 and fruiting occurred in 2014. The tree showed stunted growth, and the fruit morphology and seed morphology were differed from both the parents. This is believed to be the first successful trial on interspecific hybridization in date palm.

Keywords: *Phoenix dactylifera*, *p. pusilla*, hybridization, field evaluation.

INTRODUCTION

Date palm (*Phoenix dactylifera* L) crop improvement through breeding has been slow when compared to other crops, due to the long life cycle of date palm, 6-7 years for first flowering and slow growth habit (Simmonds, 1979). It usually takes 30 years to complete three back crosses and to obtain the first offshoots from the inter-varietal crosses. During past centuries, many new date palm inter-varietal hybrids were selected from the natural open pollinated seedling populations. The first inter varietal date palm breeding attempt on the cultivar Deglet Noor was carried out in 1912 at Arizona (Anon, 1982). Nixon and Farr started date palm breeding program at USDA during 1948 (Nixon and Far 1965; Carpenter and Reem, 1976) and their date palm breeding program was terminated in 1978 (Krueger, 1998). Inter-varietal hybridization trials were carried out in many countries for developing Boyoud resistance (Saaidi et al., 1981) and improved fruit quality (Carpenter, 1979).

In date palm cultivation, pruning, pollination, fruit thinning, bunch removal and fruit picking are highly essential for good quality fruit production. The cost of date production increases when the trees grow taller due to the high labour cost in many of date producing countries. Mechanization is also expensive and unjustifiable in the case of small growers. Frequent climbing for fruit picking is highly dangerous in the case of taller old trees. Tree height is one of the major constraints to good quality date production. In order to develop dwarf date palms, a dwarf species *Phoenix pusilla* was crossed with selected cultivars of female date palms in our Biotechnology Program of Kuwait Institute for Scientific Research (KISR). The interspecific crossing was successful, however, the hybrid embryos aborted due to poor endosperm formation during seed development. Therefore, we used *in vitro* embryo rescue technique and produced few interspecific hybrids. Details of the study are presented in this paper.

MATERIALS AND METHOD

Dwarf date palm pollen was collected from the male dwarf date palm (*Phoenix pusilla)* introduced and maintained at KISR campus (Sudhersan, 2004). The dry pollen were stored in the refrigerator for the experiments. Female date palm cultivars Barhi, Majdhool and Sultana were selected from the tissue culture date palm orchard established in 2000 at KISR campus Kuwait. During the date palm flowering season, unopened female flowers of the selected date palm cultivars were opened with a surgical knife and the *Phoenix pusilla* pollen was dusted over the female flowers and covered immediately with paper to avoid date palm pollen mixing (Figs 1,2). After the fruit set, the seed development was observed periodically by dissecting different stages of fruit development. Hybrid embryos were developed and aborted at the fruit maturity stage. Therefore, immature hybrid embryos were isolated carefully and through *in vitro* embryo rescue, hybrid plants were produced. The rooted hybrid plantlets were successfully acclimatized in a temperature and humidity controlled greenhouse, and hardened for about 6 months. Hardened interspecific hybrid plants were transferred to the field and maintained in the tissue culture date palm orchard for further field evaluation.

RESULT AND DISCUSSION

The interspecific hybridization between date palms and *Phoenix pusilla* was successful. The pollen of the *P. pusilla* affected the fruit development during the first two stages hababouk and kimri (Zaid and De Wet, 2002) and morphology at the stages of Khalal and Tamar stages. Initially fruit development was similar to normal fruit development but during later stages, fruit morphology changed. The size of fruit in Barhi was smaller than the fruit size attained by normal date palm pollen, while in the other two cultivars, Madjhool and Sultana, fruits were larger in size than the normal fruits. Previous reports on such interspecific crosses revealed that pollen from *Phoenix reclinata, P. caneriencis, P. robelenis* and *P. rupicola* crossed with date palm for the fruit quality improvement failed to produce better quality fruits, while the cross between the date palm and *P. sylvestris* produced slightly larger fruits than the normal (Nixon, 1935).

Seed development occurred at the early stages but arrested at the later stages due to less endosperm development. In the early stages, seeds showed embryo development but the embryos were aborted at the final stage. Therefore, seeds of hababouk, kimri, khalal and Rutab stages were sterilized and placed on MS basal medium with high sucrose under *in vitro* condition. Seeds from different stages showed different responses according to their stages of development. Initially, a swelling occurred at the region where the embryo is located. The seeds collected from the kimri stage fruit swelled 100 % and the others failed to swell. After two weeks, the embryo came out of the seed coat from the seeds that responded to the culture medium. The mature hybrid embryos germinated in growth hormone-free culture media.

All hybrid plantlets rescued from the embryos produced adventitious roots and elongated to about 15 cm height after 30 days in MS medium containing 0.1 mg/l NAA. All the plantlets were acclimatized to the open environmental conditions gradually.

The hardened interspecific date palm hybrids were planted in the field for experimentation. After 4 years of field growth the hybrids started producing flowers (Fids. 3, 4). Some of them were males and others were females. The female flowers were pollinated using date palm pollen and fruits were developed. The new interspecific hybrid date palm fruits were entirely different from the mother date palm in fruit colour, fruit shape, and size. The seed size and shape were also changed from the mother (Figs. 5-8). The hybrid palms are taller than the male parent and shorted than the female parent. The field evaluation, yield characteristic features and fruit quality analysis are not yet completed and are ongoing in our laboratory.

LITERATURE CITED

Anon, 1982. Date production and protection. FAO Plant production and Protection paper 35.

Carpenter, J. B. 1979. Breeding date palms in California. Ann. Rep. Date Growers' Inst. 54**:**13-14.

Carpenter, J. B. and Ream, C. L. 1976. Date palm breeding a review. Ann. Rep. Date Growers' Inst. 53**:** 25-29.

Nixon, R. W. 1985. Metaxenia and interspecific pollination in Phoenix. Proc. Am. Soc. Hort. Sci. 33**:** 21-26.

Nixon, R. W. and Far. J. R. 1965. Problems and progress in date breeding. Ann. Rep. Date Growers' Inst. 42**:** 2-5.

Krueger, R. R. 1998. Date palm germplasm: overview and utilization in the USA. Proc. of the First International. Conf. on Date Palms, Al-Ain, UAE.

Saaidi, M., Toutain, G., Bannerot, H. and Louvet, J. 1981. The selection of date palm (*Phoenix dactylifera* L.) for resistance to Boyud disease. Fr. d' Outre Mer. 35**:** 241-249.

Simmonds, M. W. 1979. Principles of crop improvement. Longman Group Ltd., London, p. 408.

Sudhersan, C. 2004. Introduction of a multipurpose palm *Phoenix pusilla* in Kuwait. Palms 48: 191-196.

Ziad, A. and P. F. De Wet. 2002. Date palm propagation. In: A. Zaid (eds), Date palm cultivation, FAO Plant Production and Protection Paper No. 156, Rome, Italy.

Figures

Fig. 1. Male Parent **Fig. 2.** Female Parent

Fig.3. Interspecific hybrid **Fig. 4.** Hybrid with fruits

Fig. 5. Fruit of male parent and hybrid **Fig. 6.** Seed of male parent and hybrid

Fig. 7. Fruit of hybrid 1 **Fig. 8.** Fruit of hybrid 2

Date Palm: Application of molecular markers

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Abstract

Molecular markers are a good way to study genetic diversity in plants, which play importance role in plant improvement programs. In date palm (*Phoenix dactylifera* L.), many molecular markers (RAPD, ISSR, SSR…) have been used for several purposes. The most important of which is the molecular identification of date palm varieties, the study of the genetic convergence between varieties, to identify resistant varieties to Bayoud disease, to verify the genetic compatibility of the vitro-plants obtained from tissue culture, in addition to these uses it was possible to determine the sex of the date palm by molecular markers. More investigations are needed to answer questions such as: Could molecular markers identify all date palm inheritance? Could molecular markers identify danger palm diseases?

Keywords: Date palm, *Phoenix dactylifera*, molecular markers, Bayoud disease, resistance, tissue culture.

INTRODUCTION

The date palm, (*Phoenix dactylifera* L.), is one of the oldest cultivated plants that it belongs to a large family of palm trees and produces dates. In Arabic, the date palm called "nakhil" and its fruits called "tamr".

The use of new biotechnology techniques in the date palm is necessary for several objectives such as: 1) The regeneration of date palm with tissue culture method: embryogenesis and organogenesis. 2) The use of molecular markersto identifyand to find the genetic relation between varieties. Many molecular marking techniques are now available,

The search for polymorphism of a genomeis a booming approach that sees each year new technique develop, each one has its advantages and disadvantages and the choice of use depends essentially on the nature of the genetic problem to betreated (Primrose et al., 2004).

The molecular markers are now becoming an essential tool for plant breeding and open new perspectives for the breeders.The most common use of molecular markers is to identify varieties. Morphological markers are essential for identifying and classifying date palm cultivars, but are not sufficient for definitive identification or classification. Currently, they have used markers (RAPD, RFLP, AFLP, ISSR and SSR) to identify and analyze the genetic diversity of date palms (Sedra et al., 1998; Zehdi et al., 2002; Al-Khalifah and Askari, 2003). However, Khierallah (2015) cited the fields of use of molecular marker for date palms.

1-The molecular markers

Molecular markers correspond to nucleotide differences existing at the level of the DNA molecule (hence the molecular term). Molecular biology techniques make it possible to reveal this sequence polymorphism. Molecular markers are used in the field of plant genome knowledge and their applications to plant breeding (De Vienne, 1990).An ideal genetic marker is:

a. Polymorphic: "genetic variability";
b. Multiallelic;

c. Codominant: the heterozygote simultaneously presents the characters of both homozygous parents; it can there forebe distinguished from each of the parental homozygotes;
d. Non-epistatic: its genotype can be "read" from its phenotype whatever the genotype at the other loci. Co-dominance and non-epistasis can be respectively defined as the absence of intra and inter locus interactions;
e. Neutral: allelic substitutions at the marker locus have no othe rphenotypic (and there fore possibly selective) effects than those that make it possible to determine its genotype. The vast majority of molecular polymorphisms are neutral;
f. Insensitive to the medium: the genotype can be inferred from the phenotype whatever the medium (De Vienne, 1998).

2- The Polymerization Chain Reaction (PCR)

PCR was described in 1985 by Kary Mullis (Haicour, 2002). The cloning of DNA fragments for sequencing purposes used to be a relatively laborious process, however, an *in vitro* technique called polymerization chain reaction (PCR) was developed to mass-produce any DNA sequence without going through cloning but using genebanks (Susan and William, 2003)

3- Determination of date palm sex

RAPD molecular markers have been used to contribute to the study of identification of male and female in date palm (*Phoenix dactylifera* L), the complexity of the date palm characterized by its dioicity,its high heterozygosity andits slow growth. It is impossible to determine the sex of the offspring of a cross at a young age, but molecular marker techniques provide tools for studying this mechanism to improve programs (Zaher and Baaziz, 2006).

4- Genetic stability

The RAPD technique wasused to compare cultured tissue grown *in vitro* from date palm with their origin 'mother plant'. Random primers were use dsuccessfully to amplify the DNA and gave sufficient polymorphism for each cultivar. The random amplified polymorphism (RAPD) technique can be applied successfully to determine genetic stability (Al-Qurainy and *al*., 2002; Eshraghi and al., 2005; Saleh and al. 2007).

5- Identification of date palm cultivars

The identification of date palm cultivars was based on morphological markers (trunk, palms and especially the fruit "dates") as well as other markers such as biochemical markers. Bennaceur et al., 1991 used Isozyme markers to study the genetic diversity of Algerian date palm cultivars. After the appearance of molecular markers, the use of markers in the identification of cultivars was very encouraging. El-Rayes (2009); Rawashdeh and al. (2006); Ben Abdallah and al., (2000) used the RAPD technique for cultivar identification. The suitability of DNA fingerprints for randomly amplified polymorphic DNA with RAPD has been examined as a genetic marker in the date palm.While Munshi and Osman (2010); Haider and al., (2012);Guettouchi and al., (2017a) combined two markers (RAPD and ISSR).

6- The use of molecular marker to identify the resistant varieties to Bayoud

Many North African plantations are destroyed by vascular Fusarium wilt (Bayoud disease), *Fusarium oxysporum*f.sp. *albedinis*,the Algerian plantations do not appear spared, they are continuously threatened by this disease due to its rapid spread in the East. This disease appeared in Morocco before 1870, in the Drâa Valley and reached in a century all Moroccan palm groves by destroying more than 12 million trees (Djerbi, 1982; Louvet et al.,1970).

The selection of quality and disease resistant varieties by conventional methods of genetics is very slow. With the new techniques, the breeder caninfer the presence of a gene by searching for the marker that is closely related to him and canthus select the resistant individuals even before the trait is expressed and in the absence of the pathogen. These markers currently give us the opportunity to scientifically judge the quality of plants and especially to identify them. Thus many strategies have been developedaiming at the molecular characterization of date palm varieties and the development of a preventive method for their protection (Baaziz, 2003).

Trifi et al., (1996) and Baaziz, (2003) used RAPD to study resistance to Bayoud disease. Two main objectives are currently targeted in all date palm breeding projects, resistance to Bayoud disease and acceptable fruit quality. Guettouchi and al.,(2017 b) used two primers for detection of the resistant of 21 varieties and 6 genotypes of Deglet Nour. They foundthat of the 21 varieties, 20 were susceptible, and only Baàrit djemal possessed mitochondria containing the R plasmid. However, the Deglet Nour variety possessed two S plasmids (373 bp fragment) in addition to an R (resistant) (265 bp fragment) plasmid and thus has the potential to be either susceptible or resistant.

CONCLUSION

Date palm is an important in the Middle East and North Africa. The identification of genetic aspect is necessary to protect this tree from threaten diseases. The most important field in which markers are used is to identify varieties, for this reason they several molecular markers (RAPD, RFLP, AFLP, ISSR and SSR). Nonetheless, the molecular markers unable to distinguish 100% between varieties, which sensitive or resistant to bayoud disease; all mutations of date palm in *in vitro* could not be distinguished, yet.

REFERENCES

Abdulla, M. and O. Gamal. 2010. Investigation on molecular phylogeny of some date palm (*Phoenix dactylifera* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia. AJCS 4(1): 23-28.

Al-Khalifah, N.S., and E. Askari. 2003. Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. Theor.Appl. Genet. 107(7) 1266-1270.

Al-Qurainy, F., F. Al-Saad, andS. Filfilan. 2002.Comparative studybetween four cultivars of date palm (*Phoenix Dactylifera* L.) producedfrom tissue culture and offshootorigins by RAPD technology. Saudi J.Biol.Sci. 9: 3-11.

Baaziz, M. 2003. Culture du palmier dattier (*Phoenix dactylifera* L.) au Maghreb et les stress pesant sur la phoenixciculture, dont la maladie du bayoud,université cadi Ayyad, Marrakech.

Ben Abdallah, A., K. Stiti, P. Lepoivre, P. Du Jardin. 2000. Identification de cultivars de palmiers dattier (*Phoenix dactylifera* L.) par l'amplification aléatoired'ADN (RAPD). Cahiers Agricultures. 9: 103-107.

Bennaceur, M., C. Lanaud, M.H. Chevalier and N.Bounagua. 1991. Genetic diversity of the date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. Plant Breed.107: 56-69.

De Vienne, D.1990.L'analyse du déterminisme génétique des caractères quantitatifs chez les végétaux. médecine/sciences.10 (6), XI-V.

De Vienne, D. 1998. Les marqueurs moléculaires en génétique et biotechnologies végétales. INRA. p 13-16.

Djerbi, M. 1991. Bilan des activités de recherche sur le Bayoud en Afrique du Nord (1989 - 1990). Rapport PNUD/FAO/RAB/88/024, FAO, Rome, Italie, 29 p.

El-Rayes, D.A., 2009. Characterization of three date palm cultivars based on RAPD Fingerprints and fruit chemical composition. JKAU: Met., Env. & Arid Land Agric. Sci.,20(2): 3-20.

Eshraghi, P., R. Zarghami, and H. Ofoghi. 2005. Genetic stability of Micropropagated plantlets in Date palm. Journal of sciences, Islamic Republic of Iran 16(4): 311-315.

Guettouchi, A., S. Elshibli, N. Haider, I. Nabulsi, N. Ykhlef. 2017a. Molecular diversity in date palm (*Phoenix dactylifera* L.) Cultivars from Ageria Indicated by RAPD and ISSR polymorphisms. Plant Cell Biotechnology and Molecular Biology. 18(1-2): 76-89.

Guettouchi A., N. Haider, I. Nabulsi, N. Ykhlef. 2017b. Molecular characterization of Algerian date palm cultivars using circular plasmid-like dans. Indian Journal of Genetics and Plant Breeding. 77(1): 170-172.

Haicour, R. 2002. Biotechnologies végétales techniques de laboratoire.Lavoisier/Tec et Doc. 328 pp.

Haider, N., I. Nabulsi, N. MirAli. 2012. Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers. Journal of Plant Biology Research. 1(2): 12-24.

Khierallah,H. S.M. 2015.Applications of molecular markers in date palm genome analysis and breeding Research. pp 47-90.*In*KsenijaTaški-Ajduković (editor), Applications of Molecular Markers in Plant Genome Analysis and Breeding. Research Signpost, Kerala, India.

Louvet, J., J. Bulit, G. Toutain, P. Rieuf. 1970. Le Bayoud, Fusariose vasculaire du Palmier dattier, symptômes et nature de la maladie , moyensde lutte. Al Awamia. 35: 161-181.

Primrose,S., R. M. Twyman, R. Old. 2004. Principes de géniegénétique. Deboeck superieur. 400 pp.

Rawashdeh I. and A.Amri. 2006. Genetic characterization of date palm varieties using RAPD markers. Jordan J. Agric. Sci. 2: 234 -241.

Saleh, M.B., M. Baum, H.S.M. Khierallah and W. Choumame. 2007. The use of RAPDs technique for the detection of genetic stability of Date Palm plantlets derived from in vitro culture of inflorescence. J. Edu. & Sic., The first conference on Biology, (September-2007).

Sedra M.H., P. Lashermes, P. Trouslot, M-C. Combes. 1998.Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica.103: 75-82.

Susan E., and S.William. 2003. Génétique. Ediscience. 490 pp.

Trifi, M., A. Benslimane, A. Rhouma, A. Rode, M. Marrakchi. 1996. Molecular characterization of Tunisian date palm varieties. 183-193.

Zaher, H. and M. Baaziz. 2006. Contribution à l'étude de l'identification des piedsmâlesetfemelles chez le palmier dattier(*Phoenix dactylifera* L.) par utilisation des marqueursmoléculaires RAPD. pp. 69-70. *In* M. Baaziz, A. Hakkou, and A. Serghini (eds.),Actes du DeuxièmeCongrès International de Biochimie. Marrakech, Morocco.

Zehdi, S., M. Trifi, A. OuldMohamed Salem, M. Marrakchi, A. Rhouma. 2002. Survey of inter simple sequence repeat polymorphisms in Tunisian date palms (*Phoenix dactylifera* L.). Journal of genetics and breeding 56:77-83.

Red Palm Weevil

Comparative susceptibilities of different life stages of the red palm weevil treated by entomopathogenic nematodes

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Abstract

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae) has become the most important pest of the date palm trees, in the world. It has been reported in the kingdom of Saudi Arabia since 1987 and in Egypt since 1992. Studies were conducted to compare preferences among RPW life stages for infection by 12 entomopathogenic nematodes (EPNs), under no choice and five stages choice experiments, and curative trials by some of EPNs isolates. All the used isolates proved to be pathogenic except the first instars of RPW larvae that were highly susceptible. However, some of the test EPNs exhibited a preference for larvae over pupae, and a lesser degree of preference for adults. In contrast some strains showed no preference for any stage. The local Egyptian isolates of EPNs were most efficient nematodes against RPW than foreign strains. But were less effective against the RPW in Saudi Arabia than in Egypt. Field assessments using trunk injection resulted in a substantial decline in the population of RPW after two successive applications within three weeks. Efficacies ranging 48-88 % was achieved in the curative assay resulting in a significant increase in palm survival compared to the untreated control. In conclusion, there is a great potential for the use of EPNs, in particular the *Steinernema sp.* (EGG4) against the RPW when injected in the date palm.

RNAi-mediated silencing of vitellogenin gene abolishes egg production in the red palm weevil, *Rhynchophorus ferrugineus* (Olivier)-A highly destructive pest of palm trees

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Abstract

The recent invasions of red palm weevil (RPW) *Rhynchophorus ferrugineus* (Oliv.) around the sphere including Saudi Arabia has become a global issue of many palm species. Principally, advanced damage results in death of the palm trees. Although, various control tactics have been applied, yet none of them seems satisfactory against this invasive pest species. Hence, exploration of the molecular approaches is awaited. We, thus, focus on silencing of the reproduction control gene vitellogenin (Vg), a major yolk protein precursor critical for oogenesis, based on RNA interference (RNAi) strategy for its possible application to control and manage the RPW population. For this, a complete *RfVg* gene transcript of 1,787 residues was isolated, sequenced and used for RNAi application. The phylogenetic analysis based on known insect Vg sequences suggested that RPW Vg has closer ancestry to other coleopterans than Vgs from other insect groups. *RfVg*-based RNAi revealed a high suppression of Vg gene expression; about 95% on day 15 of post-injection periods, which resulted in dramatically failure of Vg protein expression, atrophied ovaries or no oogenesis and ultimately no hatchability of eggs. These results suggest that knock-down of Vg gene involved in RPW reproduction has a potential to be used as a promising target for RNA-based management of RPW, a highly destructive pest of palm trees.

Red Palm weevils in Saudi Arabia and efforts to control it using genome editing with CRISPR/Cas9 technology to produce red weevil resistant (RPW) date palm

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Abstract

Biotic stresses are major constraints that limit date palm production in Saudi Arabia and in the Gulf region. Farmers always look for new varieties that can resist environmental stress and pests and produce more yields under biotic constraints such as pests. This project aims to edit/alter tolerance genes against red palm weevil (RPW), a major pest with significant yield loss in Saudi Arabian (or Middle East) date palm production. It is believed that there is a huge variation in Saudi local date palms cultivars. Recent developments in gene editing technology provide a new option for accelerated genetic improvement of the strategic food crops. Advances in genomics is identifying many gene targets that have potential to be efficiently manipulated by gene editing to deliver better adapted and more nutritious crops. This project will apply gene editing new technology CRISPR/Cas9 system to the manipulation of a range of high priority insect resistant genes in date palm. These will range from minor genetic changes aiming to alter a single nucleotide (SNP) to manipulate a simple trait that is easily measured to much larger edits that target more genetically complex traits controlling phenotypes that are more difficult to measure. The aim is to explore the practical limits of utility of this technology over this range of applications and to identify where further innovation will be required. Finally, after tackling possible obstacles the RPW candidate genes will be edited to produce nutritionally elite, RPW-resistant date palm cultivars. This project can directly lead to the elite date palms to produce healthier yield and profit for growers under agricultural and climatic constraints and pest epidemic spread. In this project, a handful of high quality popular Saudi Arabian date palm cultivars will be genetically edited to have resistance to red palm weevil (RPW) using CRISPR/Cas9 system.

Flight Activity of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) in Montenegro

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Abstract

***Rhynchophorus ferrugineus* is a highly invasive, devastating pest of palms with strong economic, environmental and social impact in every area of the presence. It is considered as the most important pest of the date palm (*Phoenix dactylifera*) in the world. *R. ferrugineus* originated from Southeast Asia from where it spread and invaded Middle East. After it was discovered in North Africa (Egypt) in 1992 and for the first time in Europe (Spain) in 1994, it has spread widely in the Mediterranean region. The pest was first detected in Montenegro in October 2012 on Canary Island date palm (*Phoenix canariensis*), the most widespread palm tree in the country. It was found in area of the city Ulcinj, placed in the most southern part of the Montenegro seacoast. After the first palm trees with umbrella-like symptom and symptoms resembling drought stress were registered and found infested, visual inspections started immediately along the whole coastal area and has been continued in the following years. Monitoring of adult flight activity has been done since 2015. Aggregation pheromone Rhylure 400 placed in white bucket traps are used. Traps are checked in two week intervals in vegetation season and once per month in winter months. Results of three years monitoring showed adults' activity during whole year with a distinct seasonal patterns in population fluctuation. Population density is very low during winter and spring months, increases in summer (June-July) up to a peak in late summer and autumn (end August- beginning November), and then decline. Rapid spreading of *R. ferrugineus* on the Montenegro seacoast resulted in devastation and death of many palms and extreme changing of our traditional coastal urban landscape.**

Keywords: monitoring, aggregation pheromone, bucket traps, Montenegro seacoast.

INTRODUCTION

Ornamental trees, shrubs and flowers as a regular part of the urban greenery, public, private gardens and parks are of huge importance for modern human life, affecting its quality of and functioning. In addition to domestic markets, international trade in ornamental plants is also a major industry, and there is ever-increasing demand for novel and high-quality products to import and export (Alford, 1995). Increased mobility and human interactions have been key drivers in the spread of alien species worldwide (Chenje and Mohamed-Katerere, 2006). The active construction works in the last 15 years, followed by numerous new buildings, along with the fast-growing touristic market in the southern part of Montenegro, were also linked with a huge importation of ornamental plants. This resulted in significantly changed appearance of the seacoast landscape, as well as in the introduction of new alien species, some of them showing drastic impact on the landscape and causing huge economic losses. The red palm weevil *Rhynchophorus ferrugineus* showed the most dramatic consequences and its spreading on the Montenegro seacoast resulted in devastation and death of many palm trees and drastic changes in the coastal landscape of our country.

R. ferrugineus originates in Southeastern Asia and Melanesia, where it is a serious pest of coconuts. From area of origin it has been advancing very rapidly westwards from mid 1980's (Ferry and Gomes, 2002). Since 1985 its spreading has been rapid in the Middle-Eastern and the Mediterranean region (Abraham et al., 2000; Faleiro et al., 2012). It reached Eastern Saudi Arabia in 1985 and from there spread to many other countries. The pest was first recorded in the Northern United Arab Emirates in 1985, from where it spread to Oman (Ferry and Gomez, 2002). In Iran it was first detected in the Savaran region in 1990 and in 1992 was discovered in Egypt (Cox, 1993). The first detection of *R. ferrugineus* in Europe was in Spain in 1994, and since then, it has spread to many European countries (Hoddle, 2015; Malumphy et al., 2016). It is primarily a pest of palms and the most important pest of date palm (*Phoenih dactylifera*) as well a serious pest of coconut (*Cocos nucifera*). As a highly invasive pest of palms *R. ferrugineus* can have a significant economic, environmental and social impact when introduced into new geographical areas. After it was introduced in the Mediterranean region the two main palm species of concern are date palm and Canary Island date palm (*Phoenix canariensis*). It also attacks some other ornamental palms such as chusan palm (*Trachycarpus fortunei*) (Malumphy et al., 2016).

R. ferrugineus was first detected in Montenegro in 2012 (Hrnčić et al., 2012). The finding was in area of the city Ulcinj on a Canary Island date palm, *Ph. canariensis*, in one hotel complex. The aim of this paper is to present results of three years monitoring of adults flight activity on the Montenegro seacoast.

MATERIALS AND METHODS

Montenegro is located in southeastern Europe (West Balkan). It has a coast on the Adriatic Sea which is 298 km long and located between 41º52` and 42º29`NGW. After the first detection of *R. ferrugineus* in Montenegro was confirmed, and the 'umbrella-like' symptom and symptoms resembling drought stress were found on palm trees together with larvae and coccons inside the infested trees, visual inspections of palms started immediately along the whole coastal area and has been continued in the following years. Apart from visual inspection, monitoring of adult fly activity started in 2015 and was carried out in three consecutive years. Aggregation pheromone Rhylure 400 (4-*methyl*-5-nonanol 90 % and 4-*methyl*-5-nonanone 10%) (Russell IPM) placed in white bucket traps is used. In 2015 traps were set up in areas of Ulcinj, Budva and Tivat; in 2016 in Ulcinj, Budva, Tivat and Herceg Novi, while in 2017 in area of Ulcinj, Bar, Budva, Tivat and Herceg Novi) (Fig. 1, Table 1). In each inspected area several sites/localities were checked. The pheromone traps were made from a 7 litre white plastic bucket with three circular holes on the lid, as well four rectangle openings (windows) cut equidistantly below the upper rim of the bucket (Fig. 2). The base of each trap was buried in the soil with the rectangle openings at the ground level, so the weevils could easily be attracted and get into the trap. Each aggregation pheromone dispenser was hung from inside the lid of the bucket with a piece of wire (Fig. 3); trap was half filled with a water in which several date fruits were added. Also, one tablespoon of liquid soap was mixed with a water to slowdown evaporation and fermentation. To ensure maximum lure longevity, traps were set up under shade, below the palm tree. Traps are checked in two week intervals during a vegetation season and ones per month in winter period. During each inspection water in the traps was changed and new dates and liquid soap replaced. Pheromones are changed in 6 week intervals.

RESULTS AND DISCUSSION

Results of three years monitoring of *R. ferrugineus* adult fly activity presented as average number of captured adults/trap/inspected area are showed in Fig. 4-6.

Our data suggest that, generally, in all inspected areas in 2015 (Fig. 4) population started build up from end of June, lasted high during summer months, with population peak reached in Ulcinj in the first decade of November. In Budva and Tivat two population peaks were reached: during mid September and in the second half of November (Budva) and mid July and first half of September (Tivat). In both areas lower population density was registered than in area of Ulcinj. Low adult capture rates was observed in winter, started from December and during spring months. In inspected areas after a short–term increasing in November, population decline.

In 2016 (Fig. 5) population started build up, depending on area, from end of June and in July and lasted high during August and September when the peak was reached in most of inspected areas. Exception was area of Ulcinj where the first peak in a season was reached in the second half of April. Depending on locality population started decreasing from mid October with a short – term increasing in November, and then decline.

Our data also showed that in 2017 (Fig. 6) population started build up, generally, in all inspected areas during June, reached the peak in Ulcinj and Bar in mid August and in Herceg Novi in September and October. In Budva and Tivat the peak was reached at the beginning of November. In all inspected areas population started decline in the second half of November.

Results of three years monitoring (2015-2017) of *R. ferrugineus* on Montenegro seacoast showed adult fly activity during a whole year, with a distinct seasonal occurence of population fluctuation. Each year population started increasing during June-July, peaked in summer and autunm (August-October) and after a short-term increasing in November, declined. Low adult capture rates was observed in winter, starting from December and during spring months. Monitoring the activity of *R. ferrugineus* is essential to protect palms against infestation and depending on mean temperatures, oviposition and egg hatching may vary in different regions (Dembilio and Jaques, 2015). Kaakeh et al. (2001) indicated two major population peak in United Arab Emirates: the first started early-March and ended mid-May; the second peak started mid-September and ended late-December. According the results obtained during the two years there are two peaks of swarming activity of *R. ferrugineus* on date palm trees throughout the year in Arement Giza governorate (Egypt): first peak occurred during March and the second during October (El-Sebay et al., 2010). Giblin-Davis et al. (2013) point out that monitoring the activity of the red palm weevil is essential for keeping a close watch on the establishment and subsequent build-up of the pest. After initial reports of infestations, it is imperative to monitor the activity of adult weevils.

Our results also showed that in pheromone traps Rhylure 400 more female were captured than males, which is in correspondence with other authors who stressed that aggregation pheromone ferrugienol attract and capture female over adult male weevils usually in ratio of two females for one male (Oehlschlager, 1998; Vidyasagar et al., 2000; Abraham et al., 2001; Faleiro, 2005).

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LITERATURE CITED

Abraham, V.A., Faleiro, J.R., Al Shuaibi, M. and Al-Abdan, S. 2001. Status of pheromone trap captured female red palm weevils from date gardens in Saudi Arabia. Journal of Tropical Agriculture. 39, 197-199.

Abraham, V.A., Mahmood, A.S., Faleiro, J.R., Abozuhairah, R.A. and Vidyasagar, P.S. 1998. An Integrated management of red palm weevil, *Rhynchophorus ferrugineus* Oliv. - A key pest of date palm in the Middle East. Sultan Qaboos Univ. J. Sci. Research, Agric. Sci. 3: 77-83.

Alford, V. D. 1995. A colour atlas of pests of ornemental trees, shrubs and flowers. London: Manson Publishing.

Chenje, M. and Mohamed-Katerere, J. 2006. Invasive species. Chapter 10 in UNEP, Africa Environment Outlook 2 –Our Environment, Our Wealth: 331-349. Nairobi, Kenya. United Nations Environment Programme. [online] http://staging.unep.org /DEWA/Africa/docs/en/aeo-2/chapters/aeo-2_ch10_INVASIVE_ALIEN_SPECIES.pdf

Cox, M. L. *1993*. Red palm weevil, *Rhynchophorus ferrugineus* in Egypt. FAO Plant Protection Bulletin, 41(1): 30–31.

Dembilop, Ó and Jaques, J.A. 2015. Chapter 2. Biology and Management of Red Palm Weevil. In: Sustainable pest Management in Date Palm: Current Status and Emerging Challenges, sustainability in Plant and Crop Protection. W.Wakil et al. (eds.). Springer International Publishing. Switzerland.

El-Sebay,Y.M.A., Abbass, M. K. and El –Shezly, M. M. 2001. Seasonal abundance and population trends of red palm weevil, Rhynchophorus ferrugineus Oliver (Coleoptera Curculioinidae). Plant Protection and Pathology, Mansoura University, Vol.1(8): 577 – 583.

Faleiro, J.R. 2005. Pheromone technology for the management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Rhynchophoridae) – A key pest of coconut (Technical Bulletin No. 4). ICAR Research Complex, Goa, 40.

Faleiro, J.R., *Ben Abdallah*, A., El-Bellaj, M., Al Ajlan, A.M. and Oihabi, A. *2012*. *Threat* of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) to Date Palm Plantations in North Africa. Arab Journal of Plant Protection, 30: 274-280.

Ferry, M. and Gómez, S. 2002. The Red Palm Weevil in the Mediterranean Area. PALMS, 46 (4): 172-178.

Giblin-Davis, R.M., Faleiro, J.R., Jacas, J.A., Peña, J.E and Vidyasagar, P.S.P.V. 2013. Chapter: 1. Biology and management of the red palm weevil, *Rhynchophorus ferrugineus*. In: Potential Invasive Pest of Agricultural Crops (ed. J. Peña). CAB International.

Hoddle, M. S. 2015. Red Palm Weevils – food or foe? Palms 59 (1): 21-30.

Hrnčić, S., Radonjić, S., Perović, T. 2012. Crveni surlaš palmi- *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) nova štetočina palmi u Crnoj Gori. Biljni lekar – Plant Doctor, 40 (6): 516-522.

Kaakeh, W., El-Ezaby, F., Aboul-Nour, M.M. and Khamis, A.A. 2001. Management of the red palm weevil, *Rhynchophorus ferrugineus* Oliv., by a pheromone/food based trapping system. In: Second international conference on date palms, 25-27 March. Al-Ain, 325-343.

Malumphy, C., Eyre, D. and Moran H. 2016. Red Palm Weevil, *Rhynchophorus ferrugineus*. Plant Pest Factsheet. Department for Environment Food & Rural Affairs,The Food and Environment Research Agency,York, UK. (online) www.planthealthportal.defra.gov.uk/assets/factsheets/Rhynchophorus-ferrugineus-Defra-PPFactsheet- Oct-2016-FINAL3.pdf

Oehlschlager, A.C. 1998. Trapping of date palm weevil. In FAO Workshop on date palm weevil (*Rhynchophorus ferrugineus*) and its control, Cairo.

Vidyasagar, P.S.P.V., Hagi, M., Abozuhairah, R.A., Al-Mohanna, O.E. and Al-Saihati,A.A. 2000. Impact of mass pheromone trapping on red palm weevil adult population and infestation level in date palm gardens of Saudi Arabia. Planter, 76, 347-355.

Table

Table 1. Areas where aggregation pheromone Rhylure 400 was set up

Area	No. of inspected sites/localities	Latitude (North)	Longitude (East)
Ulcinj	6	41º54'43''	19º14'31''
Bar	2	42º1'55''	19º8'49''
Budva	7* (6 in 2015)	42º16'53''	18º51'30''
Tivat	2* (1 in 2015)	42º26'8''	18º41'18''
Herceg Novi	2	42º26'44''	18º38'30''

Figures

Fig. 1. Map of Montenegro (seacoast is marked with oval line)

Fig. 2. Pheromone trap: 7 litre white plastic bucket with openings

Fig. 3. Aggregation pheromone dispenser hung from inside the lid of the bucket

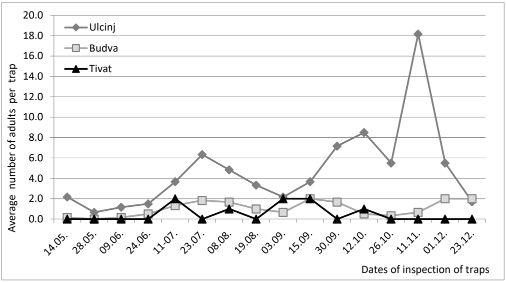

Fig. 4. Adult fly activity of *R. ferrugineus* in inspected areas in 2015

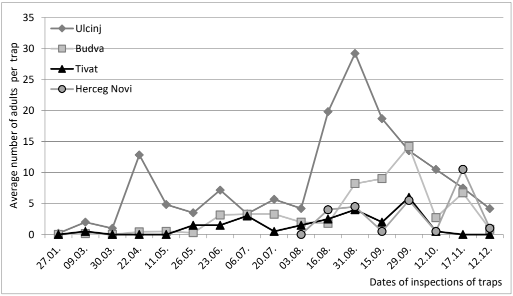

Fig. 5. Adult fly activity of *R. ferrugineus* in inspected areas in 2016

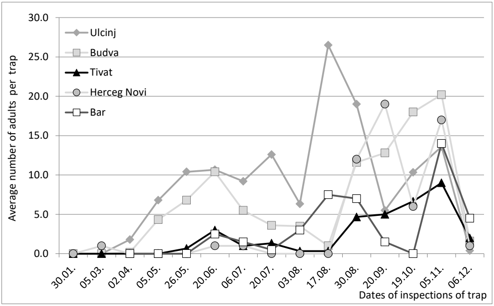

Fig. 6. Adult fly activity of *R. ferrugineus* in inspected areas in 2017

Pheromone-communication disruption through gene silencing of odorant binding and receptor proteins, a novel approach for controlling red palm weevil, *Rhynchophorus ferrugineus*

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Abstract

The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Oliver) is the most devastating quarantine pest of palm trees worldwide, was introduced to Middle Eastern countries during the 1980s. RPW has been the major date palm pest in Saudi Arabia causing heavy losses every year and its management has become the central problem for the planters. In RPWs, the perception of the environment – food, prey and mates – is guided mainly by pheromone signals, 4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferruginone). When RPWs attack a tree, individual insects are generally able to locate the tree and coordinate mass-attack with pheromone signals. We explored the RNA interference (RNAi) techniques to selectively silencing the odorant receptor (OR) and odorant binding proteins (OBPs), thus allowing us to block the pheromone communication in the adult weevil. We constructed libraries of all ORs and OBPs, selected antenna-specific and highly expressed candidates, silenced them through RNAi and sequentially presented aggregation pheromone, ferrugineol to individual RPWs and demonstrated that antenna-specific *RferOBP* and *RferOR* silencing significantly disrupt pheromone communication. The silencing of *RferOBP,* which is responsible for pheromone binding, and *RferOR*; which is responsible for pheromone detection, resulted alters RPW behavior leading to reduced pheromone detection and communication failure, as confirmed through behavioural trials and electrophysiological recordings. Considering that pheromone communication is an important aspect of *R. ferrugineus* attack on the date palm trees, where RPWs use pheromone to coordinate a mass attack that eventually leads to the death of the palm tree, silencing *RferOR* and *RferOBP* is the promising steps for the disruption of pheromone communication in *R. ferrugineus* thereby preventing the coordinated mass attack. More futuristically, the study will facilitate the development of host plant resistance mechanism and might be used to design biosensors for pheromone-based monitoring as promising approaches for controlling palm weevils.

Keywords: Red palm weevil, date palm, olfactory system, pheromone, RNAi, odorant receptor, odorant binding protein.

INTRODUCTION

The date palm is an extremely important fruit crop in Middle East countries because of its great nutritional value, high yields and long life. Saudi Arabia is the third largest producer and consumer of dates, and as per Agriculture ministry report, there are more than 15 million date palm trees in Saudi Arabia. The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is one of the most damaging invasive insect species in the world causes major economic losses in date palm trees in two decades (Faleiro, 2006;Al-Dosary et al., 2016). Given its global invasiveness and quarantine pest status, RPW has surprisingly received considerably more scientific attention in last decade (Rugman-Jones et al., 2013). In Middle Eastern countries, this pest has wreaked havoc during the last two decades, with an annual loss in the Gulf region due to the eradication of severely infested palms estimated at US $8 million in the year 2010 (Al-Dosary et al., 2016). *R. ferrugineus* is the most destructive pest of date palms in Saudi Arabia, since 1987, when the beetles surfaced in Saudi Arabia, they have infected tens of thousands ofdate palm trees, seriously threatening this vital industry, with costs of ongoing management reaching tens of millions of riyals annually (Antony et al., 2016).

In case of moderate to severe infestation, all life stages of RPW from the overlapping generations can be found inside the palm trunks and the extensive tunneling inside the trunk due to feeding by voracious grubs eventually leads to demise of the tree. The cause of the high rate of spread of this pest is human intervention *via* the transport of infested young or adult date palm trees and offshoots from contaminated areas into uninfected areas (Faleiro, 2006;Al-Dosary et al., 2016). RPW infestation is mediated primarily by their ability to locate a host, typically achieved by olfactory detection of the male aggregation pheromones (4RS,5RS)-4-methylnonan-5-ol (ferrugineol) and 4(RS)-methylnonan-5-one (ferrugineone), and this signal leads to a coordinated mass-attack that often leads to the death of the palm tree. Because of the ecological and economic effects of this pest, we selected it for study to obtain more extensive knowledge regarding its olfactory communication.

The main olfactory organ is the antenna houses sensilla and olfactory receptor neurons (ORNs). Insect pheromone reception is a complex process in which odorants penetrate the cuticle wall through multiple pores present at the surface of sensilla and reach the aqueous environment of the sensillar lymph, which is rich in soluble proteins. Pheromone detection is initiated when volatile molecule bind to odorant-binding proteins (OBPs) to cross the sensillum lymph surrounding olfactory neuron dendrites. Finally, these OBP delivers ligand molecules to the olfactory receptors (ORs)- coreceptor (Orco) located in the dendritic membranes of receptor neurons (Leal, 2013). The binding of odorant molecules by the OR–Orco receptor complex opens the channel and the chemical signal is then transformed into an electric signal that is transmitted to the brain (Benton et al., 2006). Once the signal is conveyed, the odorant degrading enzyme (ODE) protein will rapidly degrade and deactivate the odorant molecules (Leal, 2013). Malfunctioning OBPs and ORs, the essential genes in this complex olfactory system, can lead to the disablement of pheromone sensing in RPW, will ultimately help us the development of new safe pest control strategies. Gene silencing *via* RNA interference (RNAi) represents one of the possible ways in which olfactory system disruption can be achieved, and this technique is novel that can be widely used in crop protection (Baum et al., 2007;Kolliopoulou et al., 2017). We achieved this goal through RNAi gene-expression silencing mechanism that employed double-stranded RNA (dsRNA) injection to degrade specific mRNAs their by hindering RPW’s ability to sense pheromone cues and ultimately insects failed to locate hosts and mates.

MATERIALS AND METHODS

We used the RPW antennal transcriptome dataset created from our lab (Antony et al., 2016) for the data mining and identification OBPs and ORs. Total RNA collected from the antennae of *R. ferrugineus* was isolated and first-strand cDNA was synthesized. The gene-specific primers for the amplification of full-length open reading frame (ORF) of OBPs and

ORs (hereafter as '*RferOBP*' and '*RferOR'*) were designed and amplified (GenBank acc. nos., GDKA010001768, GDKA01005519).

dsRNA was synthesized from the plasmid containing the *RferOBP* and *'RferOR*. RNA synthesis was carried out following the protocols of the MEGAscript RNAi Kit (Life Technologies). Approximately 10-day-old *R. ferrugineus* pupae were used for the dsRNA experiments. Injection of 20-40 µL of 40 ng/µL dsRNA of was performed separately for each olfactory gene in the first dorsal segment of the abdomen, close to the thorax (see

https://youtu.be/j7MaoFRtjXA). Twenty-one days after injection, RNA extraction and cDNA synthesis was carried out. Gene expression in dsRNA-injected RPW was assessed for different post injection periods after injection using qRT-PCR assay. The olfactometer assay was conducted with an olfactometer unit (Volatile collection system Co., Gainesville, USA) consisting of a Y tube. The same individuals used in the olfactometer assay were tested for their response to specific stimuli using EAG. Antennae of dsRNA, nuclease free water (NFW), and no-injection (NI) RPWs were exposed to three different stimuli – (4*RS*,5*RS*)-4-methylnonan-5-ol, (Pher1) and 4(*RS*)-methylnonan-5-one (Pher2), a a kairomone, ethyl acetate (EA).

RESULTS AND DISCUSSION

We constructed libraries of all OBPs and ORs, selected antenna-specific and highly expressed candidates, silenced them through RNA interference and sequentially presented aggregation pheromones, ferrugineol, to individual RPWs and demonstrated that antenna-specific *RferOBP RferOR* involved in the first stage of ferrugineol detection (Antony et al. 2018). In *RferOBP* silencing*,* qRT-PCR gene expression data with normalization using multiple control genes (tubulin and *β*-actin) showed 99.44% and 92.77% silencing compared to NFW and NI RPWs, respectively (see Figure 1 for *RferOBP* knockdown). The qRT-PCR results revealed that there was 84.82% and 89.51% reduction in the $2^{-\Delta\Delta Ct}$ value of *RferOR* expression in dsRNA RPW compared to the NFW and NI RPWs, respectively (date not shown).

Olfactometer assays revealed significantly altered behaviors in dsRNA RPW compared to no-injection (NI) RPW; most of the control RPW adults moved towards the stimulus source, whereas only a small number of dsRNA RPW adults did so. Most of the dsRNA (*RferOBP* and *RferOR*) injected RPW adults moved away from the stimulus source (see the results: https://youtu.be/gK22G_KeTkg). To confirm the altered behaviour of the dsRNA RPW adults by olfactometer, the antennae were excised from the same individuals we used in the EAG analysis and exposed to three stimuli (Pher1, Pher2 and EA). The results revealed that *RferOBP* and *RferOR*-silenced RPWs showed significantly less in response to Pher1 than the NI control RPWs (Figure 2).

Our study successfully demonstrates that the *RferOBP* and *RferOR* genes can be silenced through RNAi technique, leading to reduced pheromone detection, thereby providing a solid baseline for further development of RPW management programs. Traditional pest management methods have proven less than satisfactory for the control of *R. ferrugineus*, leading to proposals of molecular approaches aimed at disrupting the olfactory system, given that olfaction interference has the potential to disrupt such critical behaviours as host and mate location, ultimately disturbing the reproductive process and decreasing *R. ferrugineus* populations (Antony et al., 2016;Soffan et al., 2016).

We previously reported *R. ferrugineus Orco* silencing (Soffan et al., 2016), and together with *RferOBP* and *RferOR* silencing in *R. ferrugineus via* dsRNA injection, this approach is promising for the disruption of pheromone communication in *R. ferrugineus*. To make the RNAi technique more practical for controlling *R. ferrugineus*, dsRNA delivery *via* feeding or *via* alternative delivery systems, such as the generation of transgenic plants that produce dsRNA or effective delivery systems such as synthetic nanoparticle and engineered microorganisms (Baum et al., 2007; Kolliopoulou et al., 2017), the generation of transgenic bacteria that express dsRNA (Tian et al., 2009), the chemical synthesis of siRNA (Gong et al., 2011) or the application of dsRNA in a spray form to facilitate its spread, as well as modifications that enhance its uptake by the gut and increase gene silencing efficiency, might present excellent future plans for controlling this invasive pest. Another promising area is the development of OBP-based biosensors for the detection of pheromones. Such a biotechnological applications against *R. ferrugineus* is yet to be explored, and thus our identification *RferOBP* and *RferOR* from red palm weevil holds great promise for the development of insect behavioral attractants or repellents or artificial biosensors. Considering that pheromone communication is an important aspect of *R. ferrugineus* attack of palm trees, where individual insects use male aggregation pheromone to find trees and coordinate a group attack that eventually leads to palm tree death, understanding the key OBP/OR involved in this mechanism is a significant achievement for the date palm industry.

CONCLUSION

Our study confirmed pheromone communication disruption through olfactory gene silencing and provides further evidence that RNAi application could be an attractive alternative to traditional methods for controlling *R. ferrugineus*. As *R. ferrugineus* is among the most important quarantine pest species of palm trees, and this pest has wreaked havoc in the date palm industry in Middle Eastern countries, our research findings on *R. ferrugineus* major aggregation pheromone, ferrugineol-specific *RferOBP* and *RferOR* could be used in making biosensors for pheromone-based monitoring, a novel approach, might be used in the ongoing pest management program.

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REFERENCES

Al-Dosary, N.M.N., Al-Dobai, S., and Faleiro, J.R. (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. *Emirates Journal of Food and Agriculture* 28**,** 34.

Antony, B., Soffan, A., Jakše, J., Abdelazim, M.M., Aldosari, S.A., Aldawood, A.S., and Pain, A. (2016). Identification of the genes involved in odorant reception and detection in the palm weevil *Rhynchophorus ferrugineus*, an important quarantine pest, by antennal transcriptome analysis. *BMC genomics* 17**,** 69.

Antony, B., Johny, J. and Aldosari, S. A. (2018). Silencing the odorant binding protein *RferOBP1768* reduces the strong preference of the palm weevil for the major aggregation pheromone compound ferrugineol. *Frontiers in physiology* (unpublished).

Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., and Pleau, M. (2007). Control of coleopteran insect pests through RNA interference. *Nature biotechnology* 25**,** 1322-1326.

Benton, R., Sachse, S., Michnick, S.W., and Vosshall, L.B. (2006). Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. *PLoS biology* 4**,** e20.

Faleiro, J. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *International journal of tropical Insect Science* 26**,** 135-154.

Gong, L., Yang, X., Zhang, B., Zhong, G., and Hu, M. (2011). Silencing of Rieske iron–sulfur protein using chemically synthesised siRNA as a potential biopesticide against *Plutella xylostella*. *Pest management science* 67**,** 514-520.

Kolliopoulou, A., Taning, C.N., Smagghe, G., and Swevers, L. (2017). Viral delivery of dsRNA for control of insect agricultural pests and vectors of human disease: prospects and challenges. *Frontiers in physiology* 8**,** 399.

Leal, W.S. (2013). Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annual review of entomology* 58**,** 373-391.

Rugman-Jones, P.F., Hoddle, C.D., Hoddle, M.S., and Stouthamer, R. (2013). The lesser of two weevils: molecular-genetics of pest palm weevil populations confirm *Rhynchophorus vulneratus* (Panzer 1798) as a valid species distinct from *R. ferrugineus* (Olivier 1790), and reveal the global extent of both. *PloS one* 8**,** e78379.

Soffan, A., Antony, B., Abdelazim, M., Shukla, P., Witjaksono, W., Aldosari, S.A., and Aldawood, A.S. (2016). Silencing the Olfactory Co-Receptor *RferOrco* Reduces the Response to Pheromones in the Red Palm Weevil, *Rhynchophorus ferrugineus*. *PloS one* 11**,** e0162203.

Tian, H., Peng, H., Yao, Q., Chen, H., Xie, Q., Tang, B., and Zhang, W. (2009). Developmental control of a lepidopteran pest *Spodoptera exigua* by ingestion of bacteria expressing dsRNA of a non-midgut gene. *PloS one* 4**,** e6225.

Figures

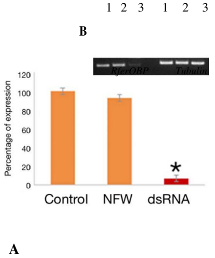

Figure 1. **A**. Normalized fold expression of dsRNA RferOBP-injected group (dsRNA) compared with no-injection (Control) and nuclease free water-injected (NFW) group. **B**. Representative visual band of the 1. Control, 2. NFW, and 3. *RferOBP* dsRNA groups (Antony *et al.,* 2018).

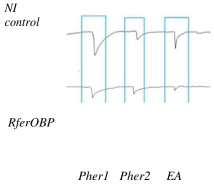

Figure 2. Electroantennography (EAG) representative waveform response of NI and dsRNA *RferOBP* injected (dsRNA) RPW to (4*RS*,5*RS*)-4-methylnonan-5-ol, (*Pher1*), 4(*RS*)-methylnonan-5-one (*Pher2*) and Ethyl acetate (*EA*) (Antony *et al.,* 2018).

Multi-use ecological biocide formulations: application to red palm weevil

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Abstract

The use of biocides in agriculture has become a common practice, even essential, to the protection of plants, vegetables and fruits, as well as to the improvement of their yield. Nevertheless, most of the active elements cause collateral damage, which is usually harmful to nature and to animals and plants health. To this end, the development of ecological biocides is crucial to food safety as well as to the preservation of the environment. The challenge remains to be, in addition to efficiency aspect, the high cost that struggles to compete with the standard chemical products. Also, we have chosen to focus our research project on agricultural protection, without any detrimental impact on man or on environment. We have hence succeeded in developing biocide formulations considered effective, and patented under the title: "Multi-usage ecological biocide formulations and process of preparation". Given the intrinsic properties of the preparation method of the ingredients, while having a good quality and cost, these ecological formulations are all indicated for applications against the Red Date Palm Weevil and against multiple families of insects in general, as well as fungi and bacteria. The formulations produced are made of natural and ecological mineral substances, such as amorphous silica and/or silicoaluminates, having a low density and a high specific surface, such as pozzolan, pyrophyllite and bentonite, etc., mixed up with active vegetal substances among the family of aromatic and medicinal plants, in the presence of other ecological ingredients. The products formulated this way, behave by dehydration, by laceration and/or intoxication or by repulsion of insects and rodents ... etc. Applied to combat the Red Palm Weevil, many developed formulations were tested by a specialized public laboratory. The results confirmed a total (100%) mortality of weevils at insect and larval stage after 4 to 7 days, which reflects the outstanding efficiency of the formulations, especially since they can be used as curative and/or preventive treatment. In conclusion, this approach can be considered as an ideal solution, simple and easy to implement, without any negative impact on health and on the environment in general.

Transcriptome analysis of fat body tissues to identify the genes responsible for red palm weevil, *Rhynchophorus ferrugineus* (Olivier), reproduction

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Abstract

In present study, the efforts were made to analyze the fat body transcriptome from the adult females with a goal to explore the genes responsible for red palm weevil (RPW) reproduction, especially the vitellogenin (*Vg*). The fat body is a dynamic tissue involved in multiple biochemical functions, including reproduction. Transcriptome analysis was performed using Illumina Hiseq 2000 next-generation sequencing (NGS) platform which resulted in a total of 43,789 unigenes (with mean lengths of 1172 bp), of which 52.93 %, 28.74%, 42.71%, 37.7%, 21.93%, and 23.52% were annotated with the non-redundant (NR), nucleotides (NT), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups (COG), and Gene Ontology (GO) databases, respectively. It was found that 20% of total unigenes were matched up to 80-100% with homologous species mainly, mountain pine beetle, *Dendroctonus ponderosae* (59.9%) and red flour beetle, *Tribolium castaneum* (26.9%). The present transcriptome analysis revealed the genes involved in different physiological functions including the genes responsible for RPW reproduction. Almost twenty-five genes annotated were involved in RPW reproduction including *Vg*, apolipophorin III, very low-density lipoprotein receptor, low-density lipoprotein receptor adapter protein 1, low-density lipoprotein receptor, and the chorion protein. The identification of reproduction control genes, especially *Vg*, will help to understand for the first time, the reproduction mechanism of the RPW at the molecular level. Moreover, the NGS data set would be a treasure for the future molecular studies and will facilitate the exploration of the biotech-based control tactics against RPW; a crucial pest oflm trees worldwide.

Eco-friendly Management of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier) in Date Palm (*Phoenix dactylifera* L.) - Seven Innovative Approaches

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Abstract

The Red Palm Weevil (RPW) (*Rhynchophorus ferrugineus* Olivier) is one of the most severe and deadly pests of date palms (*Phoenix dactylifera* L.) The Integrated Pest Management (IPM) strategies in controlling the infestation of RPW in date palm throughout the world involves surveillance, cultural control, chemical control, mass pheromone trapping, biological control, mechanical control, quarantine control etc. with limited success. Hence it is imperative to introduce new approaches for better success rates. The first proposed innovative approach may be integrated with existing IPM by covering the base of the stems with insect proof net to avoid the insects from infestation. In next innovative approach, pests and disease resistance stock and /or tissue cultures plantlets may be selected for raising seedlings in substratum in Bio-organic modified with microbial inoculants for developing new plantation to avoid infestation of RPW through good stocks and healthy growth of seedlings under field conditions. In a third approach, modified trap comprising of pheromones, light sources from solar panel fitted with a fan system may be introduced for effective trapping of insects. Yet another integrated approach could be, along with the conventional bio pesticides products, insect repellent trees, herbs and shrubs may be planted in between the rows and the plants to avoid contact of the date palm plants with RPW insects. This also provides leaf biomass through shredded leaves in the soils as well as to kill the harmful insects and pathogens by their insecticidal and antimicrobial activities. They also act as mulches crops and weed suppressive plants. In an effective approach, green cultivation techniques with bio-organic fertilizer (composed of organic fertilizers, green manure mycotrophic plants and microbial inoculants) developed in King Saud University (KSU) may be introduced to provide balanced nutrients, improve water relations, avoid soil borne pathogenic fungi, provide mulches, inoculum for next year crops etc. Another added method is mass production of entomopathogenic fungi (developed in KSU) and adding them with the bio-organic fertilizers to facilitate infection and eliminate the insects. Finally, instead of using pesticides, eco-friendly and cost effective silver nano particles (developed in KSU) may be integrated by spraying the whole plants and in addition injecting the infested plants. We hope and believe that our proposed new approaches will provide useful methods in controlling the RPW, a deadly pest in date palm.

PREAMBLE

The Red Palm Weevil (RPW) (*Rhynchophorus ferrugineus* Olivis) is one of the most severe and deadly pests of date palms (*Phoenix dactylifera* L.). The current world production of dates is reported to be seven million tones. The Integrated Pest Management (IPM) strategies in controlling the infestation of RPW in date palm throughout the world involve many different methods with limited success. To overcome the problem of RPW infestation, there is urgent need of new initiatives. The pest is distributed across Oceania, Asia, Africa and Europe (Yuezhong *et al.,* 2009) and appeared in the Middle East in the 1980s. The RPW is an internal tissue borer reported to infest 26 palm species worldwide in diverse agro-ecosystems (Malumphy & Moran, 2007). The weevil completes its life cycle within the tree trunk, destroying the vascular system and eventually causing the death of the tree. It is difficult to detect symptoms of its attack at an early stage of infestation and also to develop effective control measure. Preventive measures are thus needed for the success of any RPW-Integrated Pest Management (IPM) programme (Faleiro, 2006). Researchers across the date palm growing countries are relentlessly working in fully controlling the RPW but with limited success so far (Anonymous, 2014; Abraham et al., 1998; Ahmed et al., 2015; Faleiro, 2006; Haik, 2013; Mukhtar et al., 2010; http;//www.moa.gov.sa/alahasa-dir/portal). We are proposing some innovative approaches to upgrade the existing IPM methods for controlling the RPW effectively.

Approach I
Use insect proof net

The cultural control involves cleaning and pruning of palms trees and their offshoots to destroy the sources of infestation. This is an age-old method that many researchers followed to control of RPW and incorporated in IPM strategies. To avoid the infestation as a preventive measure, an innovative physical method may be integrated with the present proposed IPM by covering the base of the plants around the stem with insect proof nylon net. The net may be placed from 1-2 meters above the soils and buried 10 cm deep in the soil. It will prevent the insects from coming in contact with the plants and saving from infestation. Intercultural practices may be followed by removing and replacing the net. The proposed method is very simple, durable, cost effective and eco-friendly as well as easy to implement and handle along with the existing IPM.

Approach II
Growth of pest & disease free tissue culture plantlets in microbial inoculated substratum

Growth of pests and disease free healthy plant materials under laboratory conditions (tissue culture plantlets) as well as under nursery and green house conditions and/or collection of plantlets from healthy plants is a regular method of raising healthy plantation of date palm and it is also a practical approach to have healthy plantation. Pests and disease resistance stock and /or tissue cultures plantlets may be selected for raising new plantation to avoid infestation of RPW through good stocks. In the proposed approach of IPM, the plantlets may be raised in a substratum containing bio-organic fertilizers available in KSU fortified with beneficial microbial inoculants including mycorrhizal fungi to have mycotrophic plantlets which will also have beneficial microorganisms. The present proposed method is different from the conventional methods of raising seedlings under green house conditions in the growth of plantlets in bioorganic modified with microbial inoculants. The tissue culture plantlets growing in microbial inoculated fertilizer will be carrying beneficial soil microorganism that will help the plantlets in early establishment and for better growth under field conditions.

Approach III

Use modified trap

The use of pheromone traps and light traps are age-old methods that many researchers followed to control of RPW. The proposed modified trap comprises of pheromones, light sources from solar panel fitted with a fan system. In this method, innovation is in trapping of RPW by integrating the pheromones and faster trapping with fan fitted with the trapping system in same device. In our modified trap we will be using pheromones at the center of the device and around the chemical we will fit the mentioned device to attract the insects during day and night as well as with light trap during night and in both the cases the fan will suck the insects effectively from far away and put it in the container throughout 24hour period. The benefit of the modified trap over conventional traps is that the eco-friendly light sources from solar panel will be used to have light sources in the remote corner of the field.

Approach IV

Use insect repellent plants

Researchers throughout the world used different types of biopesticides in controlling the pest and diseases of crop plants with no or limited works with RPW. In a new integrated approach, along with the conventional bio pesticide products, insect repellent trees, herbs and shrubs like Neem (*Azadirahta indica)*, Mint (*Mentha*), Lemon Grass (*Cymbopogon citratus*), Lavender, Petunias, Chrysanthemums, Marigolds etc. may be planted in between the plants and rows to avoid the RPW insects coming in contact with date palm trees. The added advantage of the proposed techniques is that the insect repellent plants also provide leaf biomass through leaves shredded in the soils as well as to kill the harmful insects and pathogens by their insecticidal and antimicrobial activities. Some of the insect repellent plants also act as mulches crops and weed suppressive plants.

Approach V

Use bio-organic fertilizer for growth of date palm under field conditions

After having pest and disease free healthy planting material, the next step will be to grow plantation in organic farming systems. Bio-organic fertilizers (developed by the authors in Microbiology laboratory, Soil Science and Plant Production Department of King Saud University, Mridha and Al-Qarawi, 2013) are useful alternatives to chemical fertilizer, composed of organic matters, beneficial soil microorganisms including arbuscular mycorrhizal fungi and mycotrophic green manure legumes and non legumes plants.

The proposed green cultivation techniques with bio-organic may be incorporated with the existing IPM systems. Bio-organic fertilizer may be used in nursery conditions as well as field conditions. The microbial inoculated seedlings are transplanted to the field. The seedlings grown with bio-organic fertilizers will have different types of beneficial soil microorganisms which will reduce the transplanting shock, because inoculated seedlings will be able to uptake more water than non inoculated seedlings. The beneficial soil microorganisms will also help in up taking more nutrients (including immobile nutrients like P, Zn, B etc) in turn making the seedlings healthy. Moreover, the residual effect of nutrients for the main crops and inoculum developed during plant growth will be a source of beneficial soil inoculum for later seasons. Additional advantages of green manure plants are that the plants will provide soil coverage of the date palm to improve water relation of the main plants and reduce the growth of undesirable plants (as weed) and thereby minimize the costs of production by a considerable amount. Some of the component of bio-organic (e.g. Arbuscular Mycorrhizal Fungi and *Trichoderma* spp.) fertilizers have the capability to resist the infection of plants from soil borne pathogens (like species of *Phytophthora ,Fusarium ,Aphanomyces, Vertcillium and Sclerotium, Pythium* etc.) and some of them have the capability to decompose organic matter rapidly (e.g. *Trichoderma spp.*). These two important factors will help in growth of healthy plants that will directly or indirectly help the plants to avoid infestation of RPW under field conditions.

Approach VI

Effective mass production of entomopathogenic fungi to integrate with the proposed bio-organic

Management of RPW in date palm through eco-friendly solutions has now reached a position of pivotal significance because chemical (conventional) applications have caused serious direct environmental pollution and indirect harmful effect on human health. Biological control is one of the most important components of the management of RPW in date palm throughout the world. Out of the mentioned biological agents (fungi), *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) Sorokin is two important fungi widely used in the IPM component with limited success. The use of fungi in controlling RPW has not been found effective probably because of lack of mass production throughout the growing periods under field conditions.

Under a new approach, we are proposing to grow fungi under natural conditions to provide greater opportunity of the fungi to get in touch with insects easily. The mass production methods developed in the Microbiology Laboratory of Soil Science Department, KSU using sterilized cereals and organic matter may be introduced in the soil through proposed bio-organic fertilizers. The fungi will grow and reproduce in the soils as mycelium as well as spore inoculum and infect the insects easily and kill the insects in the soil and surrounding. The spore inoculum may also be airborne to infect the insects. The mass inoculum (mycelium and spores) of fungi may also be introduced by injecting them as solution in the infested plants as is done in case of nano solution in the present proposed IPM.

Entomopathogenic nematodes are widely regarded as being excellent biological control agents for a number of insect pests in soil and cryptic habitats. They possess many positive attributes including their wide range of hosts, safety to vertebrates, plants as well as non-target organisms, ease of in vitro production and application using standard spray equipments. Entomopathogenic nematodes may also be integrated with the proposed approaches.

Approach VII

Use eco-friendly silver nano particles

One of the most effective and widely used curative methods is the use of different types of pesticides either to prevent the infestation or to recover from the infestation. As a preventive method, the palm trees are sprayed with pesticides which may cause environmental pollution and over time may promote resistance to the pesticides in the insects. Keeping this in mind, the application of nanotechnology may be advocated in the development of efficient and potential approaches toward the management of insect pest including RPW.

The role of nanotechnology in agriculture with special reference to management of insect pests has been studied by a few researchers ((Mukhtar et al., 2010; Rai and Ingle, 2012). Fungi as an efficient mycosystem for the synthesis of metal nanoparticles were mentioned elaborately by Yadav et al., 2015. But none has used bio-synthesized nano-particles developed from fungi to control the RPW, especially entomopathogenic fungi isolated from RPW. Fungi that cause disease or infection in insects are known as entomopathogenic fungi. *Beauveria bassiana* (Balsam) and *Metarhizium anisopliae* (Metsch), two important entomopathogenic fungi, are used to manage different types of pests such as whiteflies, thrips, aphids, and red palm weevils (Thungrabeab and Tongma, 2007) etc. So far, there are no literatures available using *Beauveria bassiana* and *Metarhizium anisopliae* for the preparation of nano particles to control RPW.

Synthesized silver nano particles from entomopathogenic fungi (available in author's laboratory, Mridha and Al-Barakah, 2016) developed in King Saud University are found very effective in controlling the red palm weevil both under laboratory (from eggs to adult's weevils) and field conditions. The importance of using green nano technology over the conventional pesticide is that the nano products are environment friendly and cost effective as well as we may have safe date palm fruits for human consumption. So this innovative nano technology (silver nano particles) may be incorporated with proposed IPM in controlling RPW by spraying the plants as protective measure and injecting the plants as curative measures as a non hazardous and environmental friendly control measures.

End note

The present Integrated Pest Management strategies for controlling RPW, a destructive pest of date palm, are not very effective, environment friendly and economical. The integrated innovative approaches presented here will provide new thought and research ideas to the relevant scientist, planner, and stakeholders to overcome the problem of RPW in date palm. The new approaches are eco-friendly, cost effective and easy to implement as well as safe for human consumption of the products. A booklet on Integrated Innovative Approaches on the management of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier) in Date Palm (*Phoenix dactylifera* L.) has been published recently (Mridha and Al-Barakah, 2018b). We are confident that introducing the proposed innovative technologies for Integrated Management of Red Palm Weevil throughout the world will open a new era in controlling the destructive insects very effectively.

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REFERENCES

Abraham, V.A., Al Shuaibi, M.A., Faleiro, J.R., Abozuhairah, R.A., and Vidyasagar, P. S. P. V. (1998). An integrated approach for the management of red palm weevil *Rhynchophorus ferrugineus* Oliv. – A key pest of date palm in the middle-East. *Sultan Qaboos Univ. J. Sci. Res. (Agri. Sci.)* 3: 77-83.

Ahmed, I.A., Umma, M., Kutama, A.S., and Hassan, K.Y. (2015). Insect pests of date palm (*phoenix dactylifera* l.) and potentials of botanical insecticides for their control in the tropics: a review. *Global Adv.Res.J.Agricul.Sci.* 4(7):275-279. (Available online http://garj.org/garjas/home)

Anonymous (2014). Bio-control of Date palm Pests. ICARDA publications.

Faleiro, J.R. (2006). A review of the issues and management of red palm weevil, *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera) in coconut and date palm during the last of hundred years. *Int. J. Trop. Sci.* 26(3): 135-154.

Haik , Y. (2013). Red palm weevil sensing and control system. Patent no. US 20130199082 A1 (External Links: USPTO, USPTO Assignment, Espacenet).

Malumphy, C., and Moran, H. (2007). Red palm weevil *Rhynchophorus ferrugineus*. *Plant Pest Notice, Central Science Laboratory* (50): 1–3.

Mridha, M. A. U. and Al-Barakah, F.N. (2016a). Control of Red Palm Weevil in Date Palm with Nanoparticles using Fungi. Patent being submitted in King Saud University. Kingdom of Saudi Arabia.

Mridha, M. A. U. and Al-Barakah, F.N. (2018b) Integrated Innovative Approaches on the management of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier) in Date Palm (*Phoenix dactylifera* L.). Booklet. Plant Production Department. King Saud University.

Mridha, M.A.U and Al-Qarawi, A.A. (2013). Bio-Organic -An Effective Fertilizer for Arid Lands Agriculture and Date Palm Plantation in Saudi Arabia. *Biosci. Biotech. Res. Asia*. . 10(1): 247-251 (ISI journal).

Mukhtar, M., Rasool, K.G., Parrella, M.P., Sheikh, Q. I., Pain, A., Lopez-Llorca, L.V., Aldryhim, Y. N., Mankin R. W., and Aldawood, A. S. (2010). New Initiatives for Management of Red Palm Weevil Threats to Historical Arabian Date Palms. *Florida Entomologist*, 94(4):733-736. DOI: http://dx.doi.org/10.1653/024.094.0401

Rai, M., and Ingle, A. (2012). Role of nanotechnology in agriculture with special reference to management of insect pests. *Appl. Microbiol. Biotechnol*. 94:287–293.

Thungrabeab, M. and S. Tongma (2007). Effect of entomopathogenic fungi, *Beauveria bassiana* (balsam) and *Metarhizium anisopliae* (metsch) on non target insects. KMITL *Sci. Tech. J*. 7 (S1): 8-12.

Yuezhong L., Zeng-Rong Z., Ruiting J., Lian-Sheng W. (2009). The red plam weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), newly reported from Zhejiang, China and update of geographical distribution.- *Florida Entomologist,* 92: 386-387.

Yadav, A., Kon, K., Kratosova, G., Duran, N., Ingle, A.P., and Rai M. (2015). Fungi as an efficient mycosystem for the synthesis of metal nanoparticles: progress and key aspects of research. *Biotechnol Lett*, 37:2099–2120.

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Evaluation of the mass catching efficacy of a new trap (ELECTRAP^{TM}) for red palm weevil (*Rynchophorus Ferrugineus*) (Olivier) (Coleoptera: Curculionidae) comparing with traditional trap in date palm orchards

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Abstract

The efficacy of Electrap was evaluated to capture RPW adults under different field infestations, and a comparison of efficiency between the Electrap and the traditional traps was made. We also determined the importance of the traps in controlling management program, and the cause of effectiveness of the traps and determined male / female ratio. The experiment was conducted on 11/Sept/2017 and finished on 11/Jan/2018 (four months).The Electraps and the traditional traps were placed in four sites with high, medium and low RPW infestation. All traps were randomly placed in the middle area amongst trees, under direct sun light, keeping at least 100 meters or more between each two traps. After two months all pheromones and kairomones capsules were replaced in Electrap and traditional traps by Electrap capsules in site (A). Traps' readings were taken biweekly. Results showed the statistical superiority of the Electrap against the traditional trap under the influence of different infestation percentiles, in the four experimental sites, regardless of the level of RPW field infestation. Using the Tukey test at .05 and .01 levels, Electrap caught up to 6 times more than the traditional trap. On the other hand, traps have an important role in controlling programs as well as Electrap that six Electrap caught 549 adults during four months (89% percentile) compared to 66 adults caught by traditional trap (11% percentile) in the first experimental site A). Also, the effect of Electrap pheromones and kairomones replacement in both traps didn't significantly improve capturing of traditional trap. Pheromones and kairomones capsules should be renewed after two to three months to keep full acting potential. The female/male ratio was detected from 5.7:1 to 3:1. Finally, the theoretical calculation revealed that one Electrap may control 732 infestation spots/month, and 22thousand infestation spot/30electrap/10 hectares.

Key words: Electrap, RPW, female ratio, the traditional traps, pheromones and kairomones

INTRODUCTION

Red Palm Weevil (*Rynchophorus ferrugineus*) (Wattanapongsiri, A. 1966) is the most invasive, dangerous and deadly pest on 40 palm species (Sami Al-Saroj,2017) in most palms planted areas in the world (Abraham, et. al. 1998). South and South East Asia is the home of RPW that was considered as a major pest of coconut (Lefroy, 1906). Weak quarantine measures permitted the rapid spread of RPW to more than 50 countries (Giblin-Davis *et al.,* 2013). The RPW was reported on date palm in the Middle East during the mid 1980s (Zaid et al., 2002). Millions of RPW infested palm trees were died, with continuous losses every year and millions of USD were lost. In the GCC countries, at 1 and 5% infestation has been estimated to range from \$5.18 to \$25.92 million, respectively (El-Sabea et al., 2009). In

Jordan, which resemble a little date palm planted area, owned half million trees have lost more than 10 thousand trees and losses still happens (Mashal, M., Obaidate, B., 2015).
RPW (all stages: egg, larva, pupa, and adult) spend their lives inside the palm itself, resulting in trunk destruction then tree death (Faleiro J. R., *et al*., 2003). Adult males and females fly out of the damaged trees to find new succulent hosts, mating and depositing thousands of eggs to generate a new infestation so causing more losses (Faleiro, J.R., et al.,2011, Riley, C. V. 1894.).

The RPW females deposit about 300 eggs in separate holes on the palm trunk. Eggs hatch in 2 to 5 days into legless grubs which bore into the interior of the palms feeding on the soft succulent tissues, discarding all fibrous material. The larval period varies from 1 to 3 months. The grubs pupate in an elongate, oval, cylindrical cocoon, made out of fibrous strands. At the end of the probation period, which lasts 14 to 21 days, adult emerge and fly out of the tree searching for a new host, mating and repeating the infestation (El-Sabea, A. M. R., 2009)
Control strategies of RPW depend on monitoring, protection and treatment using an Integrated Pest Management as sanitation, cultural, mechanical, biological, physical and chemical practices (Dembilio, O. and Jacas, J. A. 2012). Monitoring is the first step in controlling programs to detect the infestation very early before causing tree destruction (Faleiro. 2006, Oehlschlager, A. C.1994), Monitoring is conducted mainly by direct Inspection of the trees or indirectly by using pheromone traps using aggregation pheromones (Hallett, R. H.1993). The trap has many functions: detecting the first entry of the insects in the orchard, detecting the population dynamics to determine the suitable time of control (Faleiro, J.R., 2006). The traps also have an important role in mass trapping (Hallett, R., *et al*. 1999) by placing lots of traps (one trap/1000m2) that capture and kill adults before mating and depositing eggs and introducing a new generation which exaggerates the infestation (Mashal, M., Obaidate, B. 2015).

Many pheromone traps were used to capture adult weevil, traditional traps (food baited traps) (Faleiro J. R. and Satarkar, V.R. 2005) as PicusanTrap^{TM} and the bucket trap, These traps consist of pheromone 625+ and kairomone capsules (Oehlschlager, A.C. 2016) fermented fruits, yeast, insecticide, water in the pail (Faleiro J. R. and Satarkar, V.R. 2005). Manipulation of the traditional traps are difficult due to continuous needed field services as frequent water, adding as well as the other components and the regular trap cleaning that is contaminated by mud, house flies and other insects and vertebrates dispersing very bad scents substances (Vacas, S., *et al*. 2013). Most of the time the traditional traps in the field become inefficient due to water drying, due to the evaporation, capsules expiring, and the attracted insects entering and getting out from the trap container without dying. On the other hand, the traditional traps used in Jordan consist of the body of the trap: 10 litres white plastic pail (with six vents, two at the lid and four on the side), one litre plastic pail placed inside the trap, the fermented fruits placed inside the small pail, pheromone lure capsule (625+) (El-Shafie, H. A. F. and Faleiro, J. R. 2017) and kairomone bottle (ethyl acetate), ten to fifteen grams of yeast, insecticide powder and water. This trap captures adults by attracting to highly strong scent dispersed from the components of the trap (Wright, R.H.1977). Adults enter through the rounded vents and then are killed by either drowning in water or by poisoning from the insecticides. There has to be a monthly renewal of Pheromones and kairomones capsules. However, renewal of water, yeast, and fermented fruits is conducted on dryness of these substances (Vacas, S., *et al*. 2013). All the more, the trap needs periodic service to be efficient all the time.

ELECTRAP (fig2) was invented to overcome all these obstacles and simplify the trap manipulation process. Once you place the traps in the orchard, there would be no need for services, except changing the pheromone and kairomone capsules after some months.

This new black trap, which has a flying saucer shape, was invented to capture RPW adults (fig3) and disable them and let them die (FIG4) using pulsed emission from MASER (Microwave Amplification by Stimulated Emission of Radiation) (Callahan, P.S. 1965, Laithwaite, E. R. 1960). Inside the Electrap device core the specially designed Phero-Kairo 925+, a pheromone lure (Ferrolure) (Oehlschlager, A.C.2016) and the formulation of the kairomone (ethyl acetate) are placed. No adding of water, insecticide, food bait, and yeast. The invention has already been granted a patent by the UAE as well as the GCC.

This experiment was conducted to evaluate the efficacy of the Electrap to capture RPW adults and to make a comparison between the Electrap and the traditional traps which are already used in Jordan and to determine the importance of traps in control management programs, also to determine the main cause of effectiveness of the traps whether it is caused by the chemical composition of the pheromone and the kairomones or caused by the composition of the trap itself, and finally to detect male/female ratio of the captured weevils.

MATERIAL AND METHOD

Experiment Sites Selecting

Four sites were selected in Jordan Valley (area infested by RPW). The sites were chosen according to the infestation density of RPW; high, medium and low, with specific conditions as follows:

- Site A - the infestation reached more than 50 % of the trees; 20 trees have got an injection treatment with many insecticide applications.
- Site B - the infestation reached more than50 % of the trees, all infested trees got an injection and fumigant treatment.
- Site C - infestation reaches more than 20% of the trees, all infested trees have got an injection and fumigant treatment for all infested trees.
- Site D - infestation less than 20% of the trees, it has got to cut and sprayed treatment.

The Experiments

The experiment was designed for the following sections to achieve the objective of the experiment

1- The efficacy of the Electrap in capturing RPW compared to traditional trap.
   This field trial part started in the four sites (A, B, C, and D) on 11Sept/2017 and finished in 11Jan/2018 (four months).
2- The efficacy of Electrap in capturing RPW compared to traditional trap using the same Pheromones and Kairomones Electrap capsules.
   This part of the trial was conducted on site A started on 29 Oct/2017, and finished on 11 Jan/2018(72 days).
3- The longevity of Electrap capsules pheromones and Kairomones.
   This part of the trial was conducted in the four sites. Started on 11Sept/2017 and finished on 11/Jan/2018 (four months).

Traps

The Electraps (FIG2) and the traditional traps (fig6) were placed in the four sites as follows:

1. Site A - six electrap + six traditional traps
2. Site B - five electrap + five traditional traps
3. Site C - three electrap + three traditional traps
4. Site D - three electrap + three traditional traps

On 11/Sept/2017 all traps were randomly placed in the middle amongst trees under direct sunlight, keeping at least 100 meters or more between each two traps to avoid scent interference. To prepare the Electrap for use, Pheromone and Kairomone capsules were placed in Electraps inside the Resonance Chamber (FIG1). These capsules can last for 3 to 6 months without renewal (company instructions). Without addition of water, insecticides or food bait inside Electraps.

The traditional traps were prepared for use in the four locations. Pheromone (625+) capsule and Kairomone (150ml of ethyl-acetate) were placed under the lid of the trap (10 liters white plastic pail) / food bait (fruits) (FIG5), 10 grams of yeast, water and insecticide were placed inside the traps. Monthly renewal of Pheromones and Kairomones capsules while water and fermented fruits are renewed when dry. On location all capsules were renewed after two months for all traps (Electraps and traditional traps).

The Data Collecting

Traps readings were taken biweekly, all captured adults were collected, taken to the laboratory and read to determine the male/female ratio. Data were gathered from 11Sept/2017 to 11Jan/2018 and analyzed by one way ANOVA analysis for Correlated Samples using Tukey Test HSD [. 05] for the. 05 level, HSD [. 01] for the.01 levels and description analysis.

RESULTS AND DISCUSSION

The efficacy of the Electrap in capturing RPW compared to the traditional traps.

The first table strongly indicates the clear differences between the average numbers of RPW caught by the Electrap and the traditional trap in the four experimental sites. Data show the superiority of the Electrap against the traditional trap under the influence of different infestation percentiles in the four experimental sites regardless to the level of RPW field infestation. This is confirmed statistically as shown in table two using Tukey test at.05 and .01 levels, between readings of Electraps and traditional traps.

The results of the statistical analysis in table two show that the Electrap captured significantly more than the traditional trap in the three sites A, B and C, whereas in the fourth site (D) the orchard infestation was almost eradicated after one month from the beginning of the experiment and the Electrap stopped capturing insects while the traditional traps did not capture any weevil in D site from the beginning.

It was noted that the capturing rate was directly proportionate to the severity of the infestation in the four experimental sites. The rate of capturing adults was highest in the infested farm, then the averages were gradually reduced to zero as at site D. So that, the traps are an important method for capturing and determining the level of field infestation, as well as in control programs.

On the other hand, the Electrap was more efficient in capturing insects than the traditional traps, (traditional traps were supplied with pheromone, kairmones, food bait, yeast, water, and pesticides while the Electrap were just supplied with pheromone and kairmones capsules). This conclusion is shown in FIG1 which represents the average mean in the four experimental sites predicting the ability of both trap types to capture the RPW adults, regardless of the level or severity of the RPW infestation in the fields or the time of reading during the year which correlated with the flight movement of the weevil inside the orchard. The graph showed a clear significant difference between the traditional trap and Electrap with the great superiority in capturing of the last which is 6 times more than the traditional trap. At the conclusion, traps have an important role in controlling program as well as Electrap as shown in FIG 3 which predicts the efficiency of the traps to capture adults RPW, It was found that six Electraps captured 549 adults during four months (89% percentile), compared to 66 adults captured by traditional traps (11% percentile) in the first experimental site (A).

Determining the source of efficiency of the Electrap which distinguishes it from the traditional trap

Figure 4 represents the experiment of replacing all the pheromone and kairomone capsules of the traditional traps and Electraps with the pheromone and kairomone capsules of the Electrap after two months from starting the experiment at the site A. Curves in Figure 4 showed the effect of this change on the trap capturing efficiency. The curves showed a rapid capturing improvement of the Electraps which continued to the end of the experiment while the capturing improvement of traditional trap was little as shown in the first reading (after interfering arrow in FIG 4). Then, the reading returns to the same level as before. This conclusion indicated that the technical operating system of the Electrap in spreading the semiochemical code through the space is specially and very efficient and not only due to the concentration and composition of pheromone and kairomone capsules (Vacas, S. *et al*. 2016). Also the mechanical capturing operation of Electrap is very efficient. In fact, once RPW adults enter into the Electrap, it could not escape due to the presence of the one-way bristles crown at the entrance. Subsequently, the trapped weevils die due to quick dehydration. Then the dead insects are removed after the traps are filled with dead weevils.

On the other hand, the principle of the operation system (abbreviated by MASER) (Porcella, L. 2013)where a fully inside mirrored ‘Resonance Chamber’ (core Electrap device), loaded by natural sunlight, incessantly reflecting the light, starts a resonance process till the saturation of the light reflection inside the chamber (Wright, R.H. 1977, Laithwaite, E. R. 1960), thereby emitting the infrared electromagnetic radio waves loaded by the lures molecules and so attracting the insects(Vacas,S. *et al*. 2016).

Effect of permanence and longevity of Pheromone and Kairmone capsules on the efficiency of the Electrap captures

FIG 5 represents the effect of renewing the capsules of pheromones and kairemone on the efficiency of RPW capture for a long run (three to six months as company instructions). The arrow in the diagram in FIG, 5 represents the interference of capsules renewal in A site while no change of capsules in B, C sites. Curve A showed the rapid improvement in Electrap capturing, while there was a dramatic decline in adult capturing as shown in B and C curves. Although, the result may be explained by the decrease in population of RPW due to continuous controlling of RPW in B and C sites, but also these same control measures were

applied on site A. However, the general trend of the curve A showed the occurrence of autumn peak of RPW population dynamics in November which is emphasized by the improvement in trap capturing in site A after renewal of the capsules. So, as a conclusion, there is a loss in the effectiveness of capsules after a maximum of two months (under high field temperatures reached more than 40 degrees Celsius and for three months under 30 degrees Celsius).
To solve the problem of the degredation of pheromone and kairomone lure after two months, the company CIQ (crop IQ TECHNOLOGY LTD) exclusively manufactured, in synergy with FIRST – UAE, a new RPW pheromone+kairomone v.41 microencapsulated IQ PHERO-KAIRO 925+ 925 mg IQ RPW lure + *(9 parts 4-methyl-5-nonanol & 1 part 4-methyl-5-nonanone)* + IQ RPW-Kairomone (Ethyl acetate - Kairomone). On January 2018, the company made the validity of the new capsules is 36 months at -2 Celsius abs the Field Efficacy of 5/6 months at +40 Celsius (Safety Data Sheet ,January 20, 2018), This capsules should be field evaluated by us as a second phase of evaluation in next months.

Sex ratio

Figure six shows, about the male/female ratio, that the female percentile was significantly higher than that of males, ranging from 5.7:1 at the beginning of the experiment to 3:1 in the end which adreed with Landolt, P.J.(1997) which coincides with the onset of winter, the increasing of males percentage with the incoming winter will encourage the mating and produce a new generation to enter the winter, On the other hand, more female captured by traps contributes more to controlling RPW than capturing males, as female mate many time with one or more male, then female lay eggs with an average of 250 eggs which will hatch to new RPW individuals. A total of 54,900 RPW could have lived and caused 54,900 infestations.

Controlling capacity

To evaluate Electrap efficiency as a part of any RPW control program, a theoretical calculation applies as follows:

- 549 total RPW captured by Electrap (fig 4)/6traps*4month = 22.875 rpw/trap/month
- 22.875 RPW/trap/month*80% females (fig 6) * 200 (eggs/adult)*40% (expected natural mortality for different insect stages) = 732 RPW individuals or infestation spot/trap/month.

  So that, if the farmer is placing 30 Electrap/10hectares (Jordan orchard unit), this will lead to a rapid decrease of the infestation as follows:
- 30*732 = 21,960 RPW individuals or infestation spots/30traps/month/10hectares.

  This 22,000 female, if not captured by the traps it will lead to 22,000 infestation spots (on the same tree or different trees) and, under suitable conditions, it will cause a disaster and outbreak of the insect population in the orchard and cause high losses within the infested and the nearby area.

  Therefore, traps as Electrap has a very essential role and strongly help in controlling weevil and it should be a part of any controlling program for RPW.

CONCLUSIONS

1. The results of the Electrap assessment and their comparison with the traditional trap show that the Electrap is very efficient in capturing RPW adults, reaching up to 6 times more efficient than the traditional trap, although the traditional trap is full supplemented by the pheromone, kairomone capsules, food bait, yeast, insecticide, and water.
2. Electrap is simply operational, easy to handle and processing.

3. Electrap is dry and does not need a continuous field service such as traditional traps that can lose any efficiency if the water dried inside.
4. Although the Electrap costs more than the traditional trap, the nature of the Electrap body makes it able to withstand the weather conditions and stay in the orchard for as long as possible. A part of the mentioned hour savings in maintenance costs.
5. It is advisable to change the pheromones and kairomones every two to three months so as not to lose the full capacity of capturing.
6. Theoretically: one Electrap may control 732 infestation spot/month, and 22 thousand infestation spot/30electrap/10 hectares.

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REFERENCES

Abraham, V.A., Al-Shuaibi, M.A., Faleiro, J.R., Abozuhairah, R.A. and Vidyasagar, P.S.P.V. 1998. An integrated management approach for red palm weevil, *Rhynchophorus ferrugineus* Oliv., a key pest of date palm in the Middle East. *Sultan Qaboos Uni. J. Sci. Res. (Agri. Sci.)*. 3: 77-83.

Al-Saoud, A. H. 2013. Effect of ethyl acetate and trap colour on weevil captures in red palm weevil (Coleoptera: Curculionidae) pheromone traps. *International Journal of Tropical Insect Science*, 33 (3):202-206.

Callahan, P.S. 1965 . Intermediate and far infrared sensing of nocturnal insects. Part I. Evidences for a far infrared (FIR) electromagnetic theory of communication and sensing in moths and its relationship to the limiting biosphere of the corn earworm, *Heliothis zea*. *Annals of the Entomological Society of America*, 58:727-45.

Dembilio, O. and Jacas, J.A. 2012. Bio-ecology and integrated management of the red palmweevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae),in the region of Valencia (Spain). *Hellenic Plant Prot. J*., 5: 1-12.

El-Sabea, A. M. R., Faleiro, J. R. and Abo El Saad. M. M. 2009. The threat of red palm weevil Rhynchophorus ferrugineus to date plantations of the Gulf region of the Middle East: an economic perspective. Outlooks on Pest Management, 20:131-134.

El-Shafie, H. A. F. and Faleiro, J. R. 2017. Optimizing components of pheromone-baited trap for the management of red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in date palm agroecosystem. *Journal of Plant Diseases and Protection* . DOI 10.1007/s41348-017-0097-5. Published on line, 26 April, 2017.

Faleiro J.R., Al-Shawaf, A.M., Al-Dandan, A.M., Al-Odhayb, A., Al-Rudayni, A., Abdallah, A.B., Peixoto, M.P., Vargas, R., Bottom, M., Chidi, S., Borges, R. and Mafra-Neto, A. 2016.Controlled Release Products for Managing Insect Pests. *Outlooks on Pest Management*, DOI: 10.1564/v27_jun_00.

Faleiro, J.R. 2006. A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *International Journal of Tropical Insect Science* 26, 135-150.

Faleiro, J.R., Abo El-Saad, M. and Al-Abbad. A. H.2011. Pheromone trap density to mass trap *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae / Rhynchophoridae /

Dryophthoridae) in date palm plantations of Saudi Arabia. *International Journal of Tropical Insect Science*, 31(1-2): 75-77.

Faleiro J. R., Rangnekar, P.A. and Satarkar, V. R. 2003. Age and fecundity of female red palm weevils *Rhynchophorus ferrugineus* (Olivier) (Coleoptera : Rhynchophoridae) captured by pheromone traps in coconut plantations of India. *Crop Protection*, 22:999-1002.

Faleiro J. R. and Satarkar, V.R. 2005. Attraction of food baits for use in red palm weevil, *Rhynchophorus ferrugineus* Olivier pheromone traps. *Indian Journal of Plant Protection* 33(1): 23-25.

Giblin-Davis, R. M., Faleiro, J. R Jacas, J. A. Peña J. E. and Vidyasagar. P.S.P.V. 2013. Coleoptera: Biology and management of the red palm weevil, *Rhynchophorus ferrugineus*. Pp. 1-34. *In* J. E. Peña [ed.], Potential Invasive Pests of Agricultural Crop Species.CABI Wallingford, UK.

Hallett, R. H., Gries, G., Gries, R., Borden, J. H., Czyzewska, E., Oehlschlager, A. C., Pierce, H. D. JR., Angerilli, N. P.D. and Rauf, A. 1993. Aggregation pheromones of two Asian palm weevils, *Rhynchophorus ferrugineus* and *Rhynchophorus vulneratus*, *Naturwissenschaften*, 80: 328-331.

Hallett, R., Oehlschlager, A. and Borden, J. 1999. Pheromone trapping protocols for the Asian palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *International Journal of Pest Management*, 45(3): 231-237.

Laithwaite, E. R. 1960. A radiation theory of the assembling of moths. *The Entomologist*, 931165): 113-117, and (1166) : 133-137.

Landolt, P.J. 1997.Sex attractant and aggregation pheromones of male phytophagous insects.*Am. Entomol.* 16:1015–1037

Lefroy, H.M. 1906. The more important insects injurious to Indian Agriculture. Govt. Press, Calcutta, India.

Mashal,M.Obaidate,B.,2015,Red palm weevil in Jordan, Book, NCARE publication funded by Buyer company 65pp.

Oehlschlager, A. C. 1994. Use of pheromone baited traps in control of red palm weevil in the kingdom of Saudi Arabia. Consultancy Report- submitted to Ministry of Agriculture, Saudi Arabia, 17pp.

Oehlschlager, A.C. 2016. Palm Weevil Pheromones- Discovery and Use. *J Chem. Ecol.,* 42(7):617-630.

Porcella, L. 2013. Electromagnetic Communication and Olfaction in Insects - Progresses in Studies and Applications on RPW Plague, http://www.uaefirst.com/electr-com-and-olfaction-in-insects.pdf, 11 pp.

Riley, C. V. 1894. Social insects from psychical and evolutional points of view. Annual address of the President of the Society, Washington, D. C. cover title Proceedings of the Biological Society of Washington, 9, 74.

Sami Al-Saroj, Emad Al-Abdallah, Abdul Moneim Al-Shawaf, Abdel Moneim Al-Dandan, Ibrahim Al-Abdullah, Abdullah Al-Shagag,Yousef Al-Fehaid, Abdallah Ben Abdallah2 And Jose Romeno Faleiro3, Efficacy of bait free pheromone trap (electraptm) for management of Red Palm Weevil, *Rhynchophorus Ferrugineus* (Olivier)(Coleoptera: Curculionidae), Pest Management In Horticultural Ecosystems,Vol. 23, No. 1 Pp 55-59 (2017)

Vacas, S., Melita, O., Michaelakis, A., Milonas, P., Roxana Minuz, R., Riolo, P., Abbass, M. K., Bue, P.L., Colazza, S., Peri, E., Soroker, V., Livne, Y., Primo, J., Navarro-

Llopis, V. 2016. Lures for red palm weevil trapping systems: aggregation pheromone and synthetic kairomone. *Pest Management Science*, 10.1002/ps.4289.

Vacas, S., Primo J. and Navarro-Llopis, V. 2013. Advances in the use of trapping systems for *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae): traps and attractant. *J. Econ. Entomol*., 106: 1739-1746.

Wright, R.H. 1977. Odour and molecular vibration: neural coding of olfactory information. *Journal of Theoretical Biology,* 64(3): 473-474.

Wattanapongsiri, A. 1966. A revision of the genera *Rhynchophorus* and *Dynamis* (Coleoptera : Cuculionidae). Bangkok, Thailand: Department of Agriculture Science Bulletin 1, 328 pp.

Zaid, A., De Wet, P. F., Djerbi, M. and Oihab, A. 2002. Diseases and pests of date palm. In: Date Palm Cultivation. Zaid, A. (Editor). FAO Plant Production and Protection Paper no. 156, Rev. 1. FAO, Rome.

Tables

Table one: Means of RPW Caught by Eectrap and Tradional Trap in The Four Experimental Sites								
Exp sites	A		B		C		D	
Date	E (M1)	T (M2)	E (M3)	T (M4)	E (M5)	T (M6)	E (M7)	T (M8)
24/09/2017	10.8	2	17	2.7	14.2	2.1	0.3	0
08/10/2017	8.8	1	14.1	1.7	11.5	1	0.3	0
15/10/2017	5.7	1.25	12.4	0.6	8.5	1	0.3	0
29/10/2017	5.8	0.2	11.5	0.6	6.8	1.1	0	0
13/11/2017	12.5	0.2	7.6	2.6	5.2	1.5	0	0
30/11/2017	12.1	4.8	7.5	1.2	4.5	1.5	0	0
15/12/2017	12.2	2.75	6.8	0.04	4.3	1	0	0
30/12/2017	11.8	1.2	4.2	0	4.1	0	0	0
11/01/2018	10.57	1.2	3.9	0	4	0	0	0

Each number represents the mean of RPW caught by six traps in site A and three traps in B, C and D experimental sites. E = Electrap – T = Traditional trap

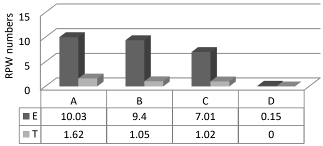
Table two: Results Of Analyzed Data In The Four Sites Using *Tukey HSD Test* [.01	
Significant AT	Non Significant at
M1 vs M2 P<.01	M1 vs M3
M1 vs M4 P<.01	M1 vs M5
M1 vs M6 P<.01	
M1 vs M7 P <.01	
M1 vs M8 P<.01	

Tukey HSD Test [. 01] =25.93

M = means; HSD = the absolute difference between any two samples means required for significance at the designated level. HSD[.01] for the .01 level.

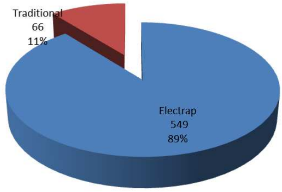
Figures

fig1:Total RPW CaughtT By Electraps and Traditional Traps in the Four Trial Sites



E = Electrap – T = traditional trap

Fig three: Total RPW Caught by both Traps during four months in A site



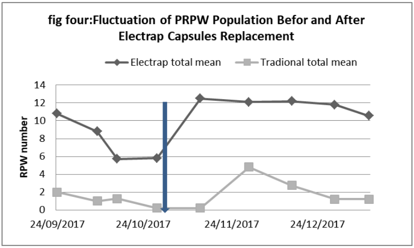


fig four:Fluctuation of PRPW Population Befor and After Electrap Capsules Replacement
Electrap total mean
Tradional total mean
RPW number
14
12
10
8
6
4
2
0
24/09/2017
24/10/2017
24/11/2017
24/12/2017

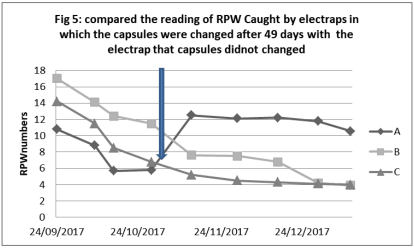
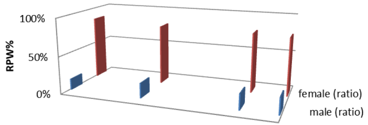


Fig 5: compared the reading of RPW Caught by electraps in which the capsules were changed after 49 days with the electrap that capsules didnot changed
RPWnumbers
18
16
14
12
10
8
6
4
2
0
24/09/2017
24/10/2017
24/11/2017
24/12/2017
A
B
C

Figure Six :Male Female Ratio Of Rpw Caugt By Traps



	29/10/2017	22/11/2017	22/12/2017	02/01/2018
male (ratio)	15%	20%	21%	22%
female (ratio)	85%	80%	79%	77%

Field evaluation to the attraction efficiency for the different sources of the red palm weevil aggregation pheromone

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Abstract

Field experiments were conducted during the periods from June, 2014 to March, 2015, in three date palm orchards located in Al Ain city, Abu Dhabi, United Arab Emirates, in order to evaluate the attraction efficacy for five different sources from the red palm weevil (RPW), Rhynchophorous ferrugineus Oliver (Coleoptera: Curculionidae), aggregation pheromone by using the standard four window black bucket trap. The three orchards are characterized by having different levels of infestation incidence by red palm weevil. The Randomize Complete Block Design (RCBD) with five treatment and three replicates was used in each of the three orchards. The aggregation pheromone sources used in this experiment were: Rhyfer 700, Pherocon RDPW Lure, Ferrugitom 700, Weevil lure, and Ferrulure +. Collectively in the three farms as well as per each farm, Weevillure aggregation pheromone trap capture significantly lower average numbers of RPW adults than Rhyfer, Pherocon, Ferrulure, and Ferrugitom pheromone sources. Rhyfer pheromone is about 1.12, 1.18, 1.56 & 1.16 % more efficient than Pherocon, Ferrugitom, Weevillure & Ferrulure, respectively.

Keywords: Aggregation pheromone sources; Date palm; Black bucket trap; Rhynchophorous ferrugineus.

INTRODUCTION

The red palm weevil (RPW), Rhynchophorous ferrugineus Oliver (Coleoptera: Curculionidae), is an economically important tissue-boring pest of date palm in many parts of the world. This weevil was first described in India as a serious pest of coconut palm (Lefroy 1906) and later on date palm (Buxton 1918). This weevil is considered the most important and major pest of date palm in the Arabian Gulf States (Abraham et al. 1998). In 1985, this weevil was accidentally introduced and established in the northern United Arab Emirates and has become widespread in the country (Ferry and Gomez 2002).

It is very difficult to detect RPW in the early stages of infestation. Generally, it is detected only after the palm has been severely damaged. The destructiveness of this weevil is abetted by several traditional farming practices, including the removal of leaves during harvesting or pruning of offshoots, which causes incidental injuries to trees. Injured trees release highly volatile compounds (kairomones) that attract male weevils (Gunawardena et al. 1998). Upon their arrival, males produce aggregation pheromones that attract both sexes, and the females begin laying eggs in soft or injured areas on the lower trunk of the tree. The newly hatched larvae feed on the soft plant tissue, digging deep into the plant trunk, compromising its structural integrity and disrupting nutrient transport to the upper part of the tree, which ultimately culminates in death of the plant if not managed (Murphy & Briscoe 1999).

Management of this weevil is by adopting an old Integrated Pest Management (IPM) strategy, which depends primarily on the use of aggregation pheromone traps for monitoring and mass trapping of the weevil adults (Abraham et al. 1998; Faleiro et al. 1998; Al-Saoud 2004; Abuagla and Al-Deeb 2012). Ferrugineol is the main aggregation pheromone of R. ferrugineus (Hallett et al.1993) and is complemented with 4-methyl-5-nonanone in mass trapping adopted in different countries (Abraham et al. 1998; Hallett et al. 1999; Vidyasagar et al. 2000).

To achieve the optimum trapping protocols it is fundamental to ensure the effectiveness of the chosen trap and aggregation pheromone to attract the weevil adults. The standard four window bucket trap is widely used, where the black colored one has been reported to be more efficient in capturing the weevil (Hallett et al. 1999; Abuagla and Al-Deeb 2012). The average recommended emission rate for the RPW aggregation pheromone is 3 mg / 24 hours (Hallett et al. 1999). While the pheromone efficiency depends on a number of factors, the most important are the composition, the concentration, the emission rate and the stability of emission, which is mainly based on the company technology in how to manufacture of the pheromone membrane that controls the rate of emission, which in turn affects the efficiency of the attractions (Kaakeh et al. 2001; Faleiro 2006). In the United Arab Emirates, the market have a number of different commercial sources of RPW weevil aggregation pheromone with variations in the membrane manufacturing technology, which is controls the pheromone emission rate, that will directly affect its efficiency in attracting the weevil. This study aims to evaluate the attracting efficiency for the five different sources from the commercial aggregation pheromone available in the local market under field conditions that represents different infestation levels by red palm weevil.

MATERIALS AND METHODS

Three private date palm orchards located in Al Ain city, Abu Dhabi Emirate, United Arab Emirates, that is having a history of infestation by the red palm weevil, were chosen to conduct this study. The first orchard located in Khatam Al Shakla with low incidence of infested date palm trees, the second orchard located in Zakher with high incidence infested date palm trees, and the third orchard located in Markhaniya with medium incidence infested date palm trees.

In each orchard five different sources from the commercial aggregation pheromone available in the local market were evaluated for its effectiveness in attraction the red palm weevil. The experimental design used in these orchards was the Randomize Complete Block (RCBD) with five treatments and three replicates. The Treatments were:

1. Rhyfer 700: 4-Methyl-5-Nonanol (9 Parts) + 4-Methyl-5-Nonanone (1 Part), Both Components 95% pure+ synergist
2. Pherocon RDPW Lure: (4-Methyl-5-Nonanol (31.5%) + 4-Methyl-5-Nonanone (3.5%)
3. Ferrugitom 700 : 4-Methyl-5-Nonanol 90%+ 4-Methyl-5-Nonanone 10%
4. Weevil lure: 4-Methyl-5-Nonanol (9 Parts) + 4-Methyl-5-Nonanone (1 Part)
5. Ferrulure +: 4-Methyl-5-Nonanol (9 parts) +4-Methyl-5-Nonanone (1 part) Plus Synergist, Pheromone is 95% Pure and synergist chemical

The trap used in the evaluation for this different sources of the aggregation pheromone is the one adopted and used by Abu Dhabi Food Control Authority (ADFCA), which is the standard four window black bucket trap, of the size 10 liters. It has four small slots on the sides and four small slots on the cover. The outer surface of the trap is coarse type that allows RPW to climb and to enter into the trap. The pheromone were installed by a metal wire in the middle of the bottom surface of the trap cover. The five pheromones sources were replaced by new ones monthly. One hundred grams from the dry unmarketable and very low quality date palm fruits were added to each trap every two weeks. In addition, five liters of water were added weekly. Total number of RPW caught by each trap was determined weekly during the period from June, 2014 to March, 2015.

Analysis of variance (ANOVA) was conducted for the data collected from 15 traps in each orchard and the means were separated by the least significant difference LSD procedure of the SAS statistical software (SAS 2001).

RESULTS

Results of this study confirm the existence of significant difference in the average number of red palm weevils caught in the three orchards. This result is compatible with the objective of the study for the evaluation of the efficiency to this different sources of the pheromone under field's conditions that represents different levels of infestation by red palm weevil (Figure 1). The average number of red palm weevils in the second orchard has reached about 279.1 weevils per trap during the study period from June 2014 until March 2015, compared with 80.2 and 43.5 weevils per trap in the third and first orchard, respectively. This indicate high differences in the total number of RPW adults collected from all the 15 traps in each orchard during the study period, that reached about 4187 weevils for the second highly infested orchard, compared to 652 weevils in first orchard that represent the low infestation level, and 1203 weevils in third orchard that represent the medium level of infestation.

Fig.1: Mean number of red palm weevil adults caught by trap in each orchard from the period June, 2014 to March, 2015. Bars labeled with different letters are significantly different ($p < 0.05$), LSD 14.50

The efficiency comparison between the different sources from the aggregation pheromone revealed significant differences in the mean number of RPW captured in each of the three orchards (Fig.2). In the first orchard having low infestation levels by red palm weevil, the mean number of RPW reached (33.33) weevils per trap by using the Weevillure pheromone source, which is significantly lower than that of the mean number (54.33) weevils per trap in the Ferrugitom pheromone. While no significant differences were found between these two sources and the other three sources, where the mean number of RPW catches reached 39.67, 39.33, and 51.0 weevils per trap for Rhyfer, Pherocon, and Ferrulure, pheromone sources, respectively. In the second orchard which is represents the high infestation incidence, the efficiency for the different sources of the pheromone seems to be compatible with the results from the first orchard. Mean number of RPW captured by the Weevillure pheromone (214.33) weevils per trap was significantly lower than that of the mean number of weevils caught by other sources. No significant differences were found between the pheromones Rhyfer and Pherocon, where the number of captured weevils reached 343 & 284 weevils per trap, as well as no significant differences were found between the number of weevils captured by using the pheromones Pherocon, Ferrugitom, and Ferrulure sources that reached (284, 275.67 & 278.67) weevils per trap, respectively (Fig.2).

The efficacy results for the different sources of the aggregation pheromone in the third orchard which is characterize by middle infestation level were also compatible with the results from the first and the second orchard. (Fig. 2). Weevillure pheromone trap captured significantly lower mean number (57.33) of RPW than Rhyfer, Pherocon and Ferrulure pheromone sources (91.33, 98.67 & 82.0) weevils per trap, respectively. However, the numbers of RPW captured by using the pheromone Ferrugitom (71.67) weevils per trap were not significant different from the mean number captured by Weevillure pheromone trap as well as not significant different from the mean number captured by Rhyfer and Ferrulure pheromone traps. Furthermore, mean number of RPW captured by using the Pherocon pheromone traps were significantly higher than that the mean number of weevils captured by using the Ferrugitom and Weevillure pheromones sources (Fig.2).

Collectively, the comparison between the means for the total numbers of RPW captured by trap in the three farms by using each of the different pheromone sources confirms the previous findings (Fig.3). Weevillure pheromone trap captured significantly lower mean number (101.67) of RPW than Rhyfer, Pherocon, Ferrugitom and Ferrulure pheromone sources (158.0, 140.67, 133.89 & 137.22) weevils per trap, respectively. No significant differences were found between the efficacy of the Rhyfer and Pherocon pheromone sources as well as between the Pherocon pheromone source in comparison with Ferrugitom and Ferrulure pheromone sources (Fig.3).

Fig: 2: Mean for number of red palm weevil caught by trap in each orchard from the period June, 2014 to

March, 2015. Bars labeled with different letters are significantly different ($p < 0.05$), LSD 18.64, 61.20, 22.39.

Fig.3: Mean number of red palm weevil caught by trap in the three orchards from the period June, 2014 to

March, 2015. Bars labeled with different letters are significantly different ($p < 0.05$), LSD 18.72.

DISCUSSION

For the most effective management of the red palm weevil, integrated pest management (IPM) was applied in most of the Gulf countries. As an important module of IPM program, the mass trapping to this weevil for over 15 years has been organized by the Ministry of Agriculture in Saudi Arabia (Abraham et al., 1998; Faleiro, 2006). Recent researches have also been focusing on finding Pheromone trapping of adult palm weevil to capture and kill the insect in order to reduce the insect populations in the field (Abraham et al. 1998; Vidyasagar et al. 2000). A total of 2,252 pheromone traps were used in the mass trapping system in an area of more than 10,000 ha in Al Qatif region, Eastern Province of Saudi Arabia (Vidyasagar et al. 2000). They reported that from an initial level of 4.12 weevils per trap per week in 1994, the adult population was reduced to 2.02 weevils per trap per week at the end of 1997. In United Arab Emirates, by using pheromone trapping in three date palm orchards during 2000 and 2001, the populations of RPW was reduced by 29.7-51.7% (Abbas et al. 2002). In Abu Dhabi Emirate, it has been reported that about two million of red palm weevil adults were trapped during the first half of the year 2013 through a project implemented by Abu Dhabi Farmers' Services Centre (ADSFC) in coordination with Abu Dhabi Food Control Authority (ADFCA), that covers about 23,050 orchards, where about 118,797 pheromone traps have been installed in these orchards (Gulf News, 2013).

In an endemic area of RPW infestation, and to reduce the weevil population in a much shorter time frame. It is recommended to increase the number of traps per unit area as well as to improve the trap efficacy in order to remove as much as from the weevil population, which will reduce the risk of new and re-infestations. In this study it is clear that the efficacy of the traps were different according to the pheromone sources. Collectively, and according to the number of RPW captured per trap in the three farms, the percentage of RPW captured by using the different sources of the pheromone were 23.6, 21.0, 19.9, 15.1 & 20.4% for the Rhyfer, Pherocon, Ferrugitom, Weevillure & Ferrulure, respectively. If we take into consideration that the IPM control program aims to remove two million from the RPW by using the trapping techniques, it means that in case of using the different sources of the pheromone, the numbers that can be collected through the use of Rhyfer, Pherocon, Ferrugitom, Weevillure & Ferrulure were 472000, 420000, 398000, 302000 &408000 weevils, respectively. From these data, we can be concluded that Rhyfer pheromone is about 1.12, 1.18, 1.56 & 1.16 % more efficient than Pherocon, Ferrugitom, Weevillure & Ferrulure, respectively.

CONCLUSIONS

The efficiency of the aggregation pheromone used by farmers to monitor and mass trapping of the red palm weevil depends on a number of factors, the most important are the composition, the concentration, the emission rate and its stability, which is mainly based on the company technology in manufacturing of the pheromone membrane, that is controls the rate of emission and its stability with the recommended life time of the pheromone, which in turn affects the efficacy of the attractions. Collectively, the comparison between the total numbers of RPW captured by the traps in the three orchards by using each of the five different pheromone sources confirms differences in the attractions efficiency according to the manufacturer's technology. Weevillure pheromone trap capture significantly lower numbers of RPW than Rhyfer, Pherocon, Ferrugitom and Ferrulure pheromone sources.

REFERENCES

Abbas M, Hanounik S, Shahdad A, Al-Bgham S. 2002. Aggregation pheromone traps, a major component of IPM strategy for the red palm weevil, Rhynchophorus ferrugineus in date palms (Coleoptera: Curculionidae). J. Pest. Sci. 79:69-73.

Abraham VA, Al-Shuaibi MA, Faleiro JR, Abozuhairah RA, Vidyasagar PSPV. 1998. An integrated Management approach for red palm weevil, Rhynchophorus Ferrugineus Olivier-A key pest of date palm in the Middle East. Agricultural Sciences, 3: 77-83.

Abuagla A M, Al-Deeb M A. 2012. Effect of bait quantity and trap colour on the trapping efficacy of the pheromone trap for the red palm weevil, Rhynchophorus Ferrugineus. J. Insect Sci. 12: 120.

Al-Saoud A. 2004. The role of Aggregation pheromone in Integrated Control of red palm weevil, Rhynchophorus Ferrugineus Olivier (Coleoptera: Curculionidae). Pages 106-112 in: Proceedings of the Date Palm Regional Workshop on Ecosystem based IPM for Date Palm in the Gulf Countries UAE University, Al-Ain, UAE: 28-30 March 2004.

Buxton P A. 1918. Report on the failure of date crops in Mesopotamia in 1918. Agric. Directorate, M. E. F. Bassarah Bull. No. 6.

Faleiro JR. 2006. A review of the issues and management of the red palm weevil Rhynchophorus Ferrugineus (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. International Journal Tropical Insect Science. 26(3): 135-154.

Faleiro JR, Abraham VA, Al-Shuaibi MA. 1998. Role of pheromone trapping in the management of Red Palm Weevil. India Coconut Journal. 29(5): 1-3. Ferry M, Gomez S. 2002. The red palm weevil in the Mediterranean area. Palms 46: 72–178.

Gunawardena N, Kern E, Kern F, Janssen E, Meegoda C, Schafer D, Vostrowski O, Bestmann H. 1998. Host attractants for red weevil, Rhynchophorus ferrugineus: Identification, Electrophysiological activity, and laboratory bioassay. J. Chem. Ecol. 24: 425-437

Gulf News, 2013. http://gulfnews.com/news/uae/environment/two-million-red-palm weevil-trapped-in-abu-dhabi-farms-during-first-half-of-2013-1.1212445.

Hallett R H, Gries G, Gries R, Borden J H, Czyzewska E, Oehlschlager A C, Pierce Jr, Angerilli N P D, Rauf A. 1993. Aggregation pheromones of two Asian palm weevils Rhynchophorus ferrugineus and R. vulneratus. Naturwissenschaften, 80: 328–331.

Hallett R H, Oehlschlager A C, Borden J H. 1999. Pheromone trapping protocols for the Asian palm weevil, Rhynchophorus ferrugineus (Coleoptera: Curculionidae). International Journal of Pest Management, 45, 231–237

Kaakeh W, El-Ezaby F, Aboul-Nour M. M, Khamis A. 2001. Management of the red palm weevil, Rhynchophorus Ferrugineus Olivier, by a pheromone /food-based trapping system. Pages 325–343: In Proceedings of the Second International Conference on Date Palms, March 2001, Al-Ain, UAE. UAE University, Al-Ain, United Arab Emirates.

Lefroy HM. 1906. The more important insects injurious to Indian agriculture. Govt. Press, Calcutta.

Murphy ST, Briscoe BR. 1999. The red palm weevil as an alien invasive: biology and prospects for biological control as a component of IPM. Biocontrol News and Information, 20:35–45SAS Institute. SAS Users Guide, Release 8.0 ed. SAS Institute; 2001.

Vidyasagar P S, Abozuhairah R A, Rai-Mohanna OE, Al-Saihati AA. 2000. Impact of mass pheromone trapping on red palm weevil adult population and infestation level in date palm gardens of Saudi Arabia. Planter, 76: 347–355.

Figures

Fig.1: pheromone and kairomone capsules mounted in Electrap Resonance Chamber

Fig. 2: the Electrap body

Fig.3:: landing of RPW on Electrap cascade

Fig.4: RPW caught inside the Electrap

Fig.5:: preparation of traditional trap

Fig.6: tradirional trap setting

Fig.7: contaminated traditional trap after two weeks from setting

Pests and Diseases of Date Palm

Sustainable date palm production and bio pesticide research

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Abstract

The date palm *Phoenix dactylifera* has played an important role in the day-to-day life of the people for the last 7000 years. Today worldwide production, utilization and industrialization of dates are continuously increasing since date fruits have earned great importance in human nutrition owing to their rich content of essential nutrients. Date palm, *Phoenix dactylifera* L., is one of the oldest fruit trees in the world. The date palm is believed to have originated in the lands around the Persian Gulf and in ancient times was especially abundant between the Nile and Euphrates rivers. The date has been traditionally a staple food in Algeria, Morocco, Tunisia, Egypt, the Sudan, Arabia and Iran. The founder of UAE attached a great importance to agricultural development in general, and to the date palm in particular. This attention is clearly evident in the fast growth in the number of palm trees in the continued increase in the size and variety of date products, in the extensive use of modern technologies, and in the important initiatives undertaken in the areas of manufacturing and marketing of date fruit. The number of the date palms is about 100 million worldwide, of which 62 million palms can be found in the Arab world. (A.Zaid).

Keywords: *Phoenix dactylifera*, Qur'anic and Prophetic citations, Bio pesticide, Rhyncophorus ferruginous Oliver; Chromatographic fingerprinting;

Historical overview of dates (phoenix dactylifera):

Dates (Phoenix dactylifera) are one of the members of the palm family Arecaceae, or Palmae [Zohary, 1993]. The species name dactylifera "date-bearing" originate from two words; one from Greek dáktulos "date" [δάκτυλος.] and the stem of the Greek verb ferō [fĕro.]. The date palm (Phoenix dactylifera L.) is one of oldest cultivated plants of human kind and used as food for 6000 years [Sulieman A,2012].

Date palm, *Phoenix dactylifera* L., is one of the oldest fruit \trees in the world. The date palm is believed to have originated in the lands around the Persian Gulf and in ancient times was especially abundant between the Nile and Euphrates rivers. The date has been traditionally a staple food in Algeria, Morocco, Tunisia, Egypt, the Sudan, Arabia and Iran. The founder of UAE attached a great importance to agricultural development in general, and to the date palm in particular. This attention is clearly evident in the fast growth in the number of palm trees in the continued increase in the size and variety of date products, in the extensive use of modern technologies, and in the important initiatives undertaken in the areas of manufacturing and marketing of date fruit. The number of the date palms is about 100 million worldwide, of which 62 million palms can be found in the Arab world. (A.Zaid).

The Date Palm is recorded in ancient history extending over an area from the Indus Valley (now Pakistan) to Mesopotamia (now Iraq), the Nile Valley, Southern Persia, Eastern Mediterranean and the Horn of Africa. Historic records however, tend to describe the fruit without being able to say whether it is derived from a plant of cultivation or from a wild plant. Archaeobotanical evidence would have a difficult task to establish whether seed or other parts of a date palm were from domesticated or wild plants. References to the Date in the Nile Valley and Tigris/Euphrates valleys suggest it was a plant of cultivation along with other early developments in agriculture as it was found in an area where agriculture had been practiced for 5 millennia.

Such a wide distribution implies that *Phoenix dactylifera* either evolved as a plant covering quite extensive geographic, soil and climatic conditions, which is unlikely, or it spread with the help of man after originating in a more limited geographic region. I support the theory of the Date being of Indus Valley origin (Geoffrey Bibby, "Looking for Dilmun"). Within the Indus Valley is found a sister specie, *Phoenix sylvestris*, (Sugar Date Palm, or Toddy Palm) which still occurs in the wild; its sap is used to produce a crude sugar.

Phoenix dactilyfera most likely grew wild in the Indus Valley as a natural hybrid of *P.sylvestris* where it was appreciated as a wild fruit and probably cultivated as early as the 6th millennia BC. Supposed wild occurrences of *P.dactylifera* in other locations is more likely to be man assisted, i.e. discarding of seed along trade routes and surrounding trade centres. Confirming the wild source of Phoenix dactylifera would make fascinating research.

From 5000 BC there have been finds of Date Palm seeds in association with human settlement. The oldest radiocarbon dated discovery of Date seeds was on Dalma island, part of the Abu Dhabi Islands group. Two seeds were found in 1998, the oldest was 5110 BC and the other, 4670 BC. As there was no evidence of cultivation of Date Palms in the region at that time, it is probable that these seeds came from traders.

The earliest evidence of Date Palm cultivation was during the Eridu period in Lower Mesopotamia. Date seeds were also found in the third millennia BC in the 'Royal Cemetery" at Ur (Ellison et al. 1978: 171-2) but their origin is not established. The Sumerian words for date (zulum) and date palm (gishimmar) belonged to a group of words considered by Sumerologist Benno Lands Berger to be non-Sumerian loan words from a hypothetical pre-Sumerian language associated with a pre Sumerian aboriginal population. This suggests that The Date was known before the Sumerians and maybe it was an indigenous plant in that region.

Date Palm Biology

Largely restricted to plantations, today reproduction in the date palm is largely artificial. The ratio of female to male plants in such plantations is often as much as 50 to 1, and pollen grains are collected from male plants and transferred by hand or blown by spray machines onto female plants. Natural pollination is achieved with the aid of insects or the wind, with the fruits taking six to eight months to ripen (Rieger, M. 2006). The date palm begins to bear fruit at three to five years of age, becoming fully mature at 12 years (UC Davis, 2010). A date palm can live for up to 150 years.

Quranic References for Date Palm

Date palm are described more than 20 times in holy Qur'an; 8 times the date-palm is mentioned alone; And at 12 places it is clubbed with other fruits like olive, pomegranate and grapes.

- SURA II (Baqara the Heifer), V : 266
- SURA VI (An'am-Cattle), V: 99
- SURA VI (An'am-Cattle), V: 141

- SURA XIII (Raad-Thunder), V: 4
- SURA XVI (Nahl-The Bee), V: 11
- SURA XVI (Nahl-The Bee), V: 67
- SURA XVII (Bani Israel), V: 91
- SURA XVIII (Kahaf-TheCave), V: 32
- SURA XIX (Maryam-Mary), V: 23
- SURA XIX (Maryam-Mary), V: 25
- SURA XX (TaHa –Mystic letter) V: 71
- SURA XXIII (Muminun-The Believers) V: 19
- SURA XXVi (Shura-The Poet) V: 148
- SURA XXXVI (Yasin-The Abbreviated letters) V: 10
- SURA L (Qaf- The Abbreviated letters) V: 34
- SURA LIV (Qamar-The Moon) V: 20
- SURA LV (Rahman-Allah most gracious) V: 11
- SURA LV (Rahman-Allah most gracious) V: 68
- SURA LXIX (Haqqa –The Sure Reality) V: 7
- SURA LV (Abasa -He Frowned) V: 29

<u>SURAH (Baqara the Heifer), V: 266</u>

أَيَوَدُّ أَحَدُكُمْ أَن تَكُونَ لَهُۥ جَنَّةٌ مِّن نَّخِيلٍ وَأَعْنَابٍ تَجْرِى مِن تَحْتِهَا
ٱلْأَنْهَـٰرُ لَهُۥ فِيهَا مِن كُلِّ ٱلثَّمَرَٰتِ وَأَصَابَهُ ٱلْكِبَرُ وَلَهُۥ ذُرِّيَّةٌ ضُعَفَآءُ
فَأَصَابَهَآ إِعْصَارٌ فِيهِ نَارٌ فَٱحْتَرَقَتْ ۗ كَذَٰلِكَ يُبَيِّنُ ٱللَّهُ لَكُمُ ٱلْـَٔايَـٰتِ لَعَلَّكُمْ
تَتَفَكَّرُونَ ﴿٢٦٦﴾

[266]*Would any one of you like it that he would have a garden of dates and grapes beneath which rivers would have been flowing; for him, there is every kind of fruit in it, and old age came to him and he has weak children, then came a whirlwind on it in which there was fire, so it was burnt? Thus Allah explains to you His signs, so that you may ponder.*

SURAH Al, An’am V-99

وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦ نَبَاتَ كُلِّ شَىْءٍ فَأَخْرَجْنَا
مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ ٱلنَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ
وَجَنَّٰتٍ مِّنْ أَعْنَابٍ وَٱلزَّيْتُونَ وَٱلرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَٰبِهٍ ۗ ٱنظُرُوٓا۟
إِلَىٰ ثَمَرِهِۦٓ إِذَآ أَثْمَرَ وَيَنْعِهِۦٓ ۚ إِنَّ فِى ذَٰلِكُمْ لَءَايَٰتٍ لِّقَوْمٍ يُؤْمِنُونَ ﴿٩٩﴾

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[99] And it is He who has sent down water from the heaven, then We brought forth every things of growth and out of it We brought forth the green (foliage) from which We bring forth-clustered grains and out of the sheaths of palms the bunches close to one another and gardens of grapes and olives and pomegranates, similar in some respect and dissimilar in some other respects. Look at its fruit when it bears fruit and its ripening. Undoubtedly there are signs in it for the persons believing.

SURAH Al, An’am V-141

۞ وَهُوَ ٱلَّذِىٓ أَنشَأَ جَنَّٰتٍ مَّعْرُوشَٰتٍ وَغَيْرَ مَعْرُوشَٰتٍ وَٱلنَّخْلَ
وَٱلزَّرْعَ مُخْتَلِفًا أُكُلُهُۥ وَٱلزَّيْتُونَ وَٱلرُّمَّانَ مُتَشَٰبِهًا وَغَيْرَ
مُتَشَٰبِهٍ ۚ كُلُوا۟ مِن ثَمَرِهِۦٓ إِذَآ أَثْمَرَ وَءَاتُوا۟ حَقَّهُۥ يَوْمَ حَصَادِهِۦ ۖ
وَلَا تُسْرِفُوٓا۟ ۚ إِنَّهُۥ لَا يُحِبُّ ٱلْمُسْرِفِينَ ﴿١٤١﴾

[141] It is He Who produced gardens, with trellises and without, and dates, and tilth with produce of all kinds, and olives and pomegranates, similar (in kind) and different (in variety): eat of their fruit in their season, but render the dues that are proper on the day that the harvest is gathered. But waste not by excess: for Allah loveth not the wasters.

SURAH XIII (Ra’d-Thunder) V: 4

وَفِى ٱلْأَرْضِ قِطَعٌ مُّتَجَٰوِرَٰتٌ وَجَنَّٰتٌ مِّنْ أَعْنَٰبٍ وَزَرْعٌ وَنَخِيلٌ
صِنْوَانٌ وَغَيْرُ صِنْوَانٍ يُسْقَىٰ بِمَآءٍ وَٰحِدٍ وَنُفَضِّلُ بَعْضَهَا عَلَىٰ بَعْضٍ فِى
ٱلْأُكُلِ ۚ إِنَّ فِى ذَٰلِكَ لَءَايَٰتٍ لِّقَوْمٍ يَعْقِلُونَ ﴿٤﴾

[4] *And within the land are neighboring plots and gardens of grapevines and crops and palm trees, [growing] several from a root or otherwise, watered with one water; but* We *make some of them exceed others in [quality of] fruit. Indeed in that are signs for a people who reason.*

SURAH XVI (Nahl- The Bee) V: 11

يُنۢبِتُ لَكُم بِهِ ٱلزَّرۡعَ وَٱلزَّيۡتُونَ وَٱلنَّخِيلَ وَٱلۡأَعۡنَٰبَ وَمِن
كُلِّ ٱلثَّمَرَٰتِۚ إِنَّ فِى ذَٰلِكَ لَأٓيَةٗ لِّقَوۡمٖ يَتَفَكَّرُونَ ١١

[11] *He causes to grow for you thereby the crops, olives, palm trees, grapevines, and from all the fruits. Indeed in that is a sign for a people who give thought.*

SURAH XVI (Nahl – The Bee) V: 67

وَمِن ثَمَرَٰتِ ٱلنَّخِيلِ وَٱلۡأَعۡنَٰبِ تَتَّخِذُونَ مِنۡهُ سَكَرٗا وَرِزۡقًا حَسَنًاۚ إِنَّ فِى
ذَٰلِكَ لَأٓيَةٗ لِّقَوۡمٖ يَعۡقِلُونَ ٦٧

[67] *And from the fruits of the palm trees and grapevines you take intoxicant and good provision. Indeed in that is a sign for a people who reason.*

SURAH XVII (Bani-Israel-The Children of Israel) V: 91

أَوۡ تَكُونَ لَكَ جَنَّةٞ مِّن نَّخِيلٖ وَعِنَبٖ فَتُفَجِّرَ ٱلۡأَنۡهَٰرَ خِلَٰلَهَا
تَفۡجِيرًا ٩١

[91] *Or [until] you have a garden of palm trees and grapes and make rivers gush forth within them in force [and abundance].*

SURAH XVIII (Kahf-The Cave). V: 32

۞وَٱضۡرِبۡ لَهُم مَّثَلٗا رَّجُلَيۡنِ جَعَلۡنَا لِأَحَدِهِمَا جَنَّتَيۡنِ مِنۡ أَعۡنَٰبٖ
وَحَفَفۡنَٰهُمَا بِنَخۡلٖ وَجَعَلۡنَا بَيۡنَهُمَا زَرۡعٗا ٣٢

[32]*And present to them an example of two men: We granted to one of them two gardens of grapevines, and We bordered them with palm trees and placed between them [fields of] crops.*

SURAH XIX (Maryam-Mary) V: 23

The importance of dates has been documented in the Qur'an in Surah Maryam.
One significant role of dates comes as when Mary gave birth to the Prophet Jesus (may peace be upon him) under a palm tree, she heard a voice telling her:

فَأَجَآءَهَا ٱلۡمَخَاضُ إِلَىٰ جِذۡعِ ٱلنَّخۡلَةِ قَالَتۡ يَٰلَيۡتَنِي مِتُّ
قَبۡلَ هَٰذَا وَكُنتُ نَسۡيٗا مَّنسِيّٗا ﴿٢٣﴾

SURAH XIX (Maryam-V-23)

[23]Then the pains of the childbirth brought her to the trunk of a palm-tree. She said, Oh, would that I had died before this and had become a thing forgotten, lost'.

وَهُزِّيٓ إِلَيۡكِ بِجِذۡعِ ٱلنَّخۡلَةِ تُسَٰقِطۡ عَلَيۡكِ رُطَبٗا جَنِيّٗا
﴿٢٥﴾

[25]And shake towards yourself holding the trunk of the palm tree, fresh and ripe dates will fall upon you.

فَكُلِي وَٱشۡرَبِي وَقَرِّي عَيۡنٗاۖ فَإِمَّا تَرَيِنَّ مِنَ ٱلۡبَشَرِ
أَحَدٗا فَقُولِيٓ إِنِّي نَذَرۡتُ لِلرَّحۡمَٰنِ صَوۡمٗا فَلَنۡ أُكَلِّمَ ٱلۡيَوۡمَ
إِنسِيّٗا ﴿٢٦﴾

[26] *Then eat and drink and Cool your eyes; then if you see any man, then say, 'I have vowed a fast to the Most Affectionate, so I shall not speak to anyone today'.*

SURAH XX (Ta-Ha-Mystic Letter, T.H.) V: 71

قَالَ ءَامَنتُمۡ لَهُۥ قَبۡلَ أَنۡ ءَاذَنَ لَكُمۡۖ إِنَّهُۥ لَكَبِيرُكُمُ ٱلَّذِي
عَلَّمَكُمُ ٱلسِّحۡرَۖ فَلَأُقَطِّعَنَّ أَيۡدِيَكُمۡ وَأَرۡجُلَكُم مِّنۡ خِلَٰفٖ وَلَأُصَلِّبَنَّكُمۡ
فِي جُذُوعِ ٱلنَّخۡلِ وَلَتَعۡلَمُنَّ أَيُّنَآ أَشَدُّ عَذَابٗا وَأَبۡقَىٰ ﴿٧١﴾

[71]Firawn [*Pharaoh*] *said, "You believed him before I gave you permission. Indeed, he is your leader who has taught you magic. So I will surely cut off your hands and your feet on opposite sides, and I will crucify you on the trunks of palm trees, and you will surely know which of us is more severe in [giving] punishment and more enduring."*

SURAH XXIII (Mu-minun- The Believers) V: 19

فَأَنشَأْنَا لَكُم بِهِۦ جَنَّـٰتٍ مِّن نَّخِيلٍ وَأَعْنَـٰبٍ لَّكُمْ فِيهَا فَوَٰكِهُ كَثِيرَةٌ
وَمِنْهَا تَأْكُلُونَ ﴿١٩﴾

[19]*And We brought forth for you thereby gardens of palm trees and grapevines in which for you are abundant fruits and from which you eat.*

SURAH XXVI (Shu'araa – The Poets) V: 148

وَزُرُوعٍ وَنَخْلٍ طَلْعُهَا هَضِيمٌ ﴿١٤٨﴾

[148] And fields of crops and palm trees with softened fruit?

SURAH XXXVI (Ya-sin- Abbreviated Letters) V: 34

وَجَعَلْنَا فِيهَا جَنَّـٰتٍ مِّن نَّخِيلٍ وَأَعْنَـٰبٍ وَفَجَّرْنَا فِيهَا مِنَ ٱلْعُيُونِ
﴿٣٤﴾

[34] *And We placed therein gardens of palm trees and grapevines and caused to burst forth there from some springs.*

SURAH L. (Qaf- Abbreviated Letters) V: 10

وَٱلنَّخْلَ بَاسِقَـٰتٍ لَّهَا طَلْعٌ نَّضِيدٌ ﴿١٠﴾

[10] *And lofty palm trees having fruit arranged in layers .*

SURAH LIV (Qamar-The Moon) V: 20

تَنزِعُ ٱلنَّاسَ كَأَنَّهُمْ أَعْجَازُ نَخْلٍ مُّنقَعِرٍ ﴿٢٠﴾

[20]*Extracting the people as if they were trunks of palm trees uprooted.*

<u>SURAH LV (Rahman-Allah-Most Gracious) V: 11</u>

فِيهَا فَٰكِهَةٌ وَٱلنَّخۡلُ ذَاتُ ٱلۡأَكۡمَامِ ١١

[11]*There is fruit and palm trees having sheaths [of dates].*

<u>SURAH LV (Rahman-Allah-Most Gracious) V: 68</u>

فِيهِمَا فَٰكِهَةٌ وَنَخۡلٌ وَرُمَّانٌ ٦٨

[68] *In both of them are fruit and palm trees and pomegranates.*

<u>SURAH LXIX (Haqqa- The Sure Reality) V: 7</u>

سَخَّرَهَا عَلَيۡهِمۡ سَبۡعَ لَيَالٍ وَثَمَٰنِيَةَ أَيَّامٍ حُسُومًا فَتَرَى ٱلۡقَوۡمَ فِيهَا صَرۡعَىٰ
كَأَنَّهُمۡ أَعۡجَازُ نَخۡلٍ خَاوِيَةٍ ٧

[7]*Which Allah imposed upon them for seven nights and eight days in succession, so you would see the people therein fallen as if they were hollow trunks of palm trees.*

<u>SURAH LXXX (Abasa-He-Frowned) V: 29</u>

وَزَيۡتُونًا وَنَخۡلًا ٢٩

[29] And *the olive and the date palm.*

Other Qur'anic Names

Apart from the reference of date-palm under the name of Nakhl, it has been mentioned by other names too. For instance:

1. It's particular variety is referred to as Leenat in chapter 59 (Surah) Hashr, verse no. 5.
2. It is mentioned as Naqir in chapter 4 (Surah) Nisa, verse no. 53 and 124.
3. Qitmir in chapter 35 (Surah) Fatir, verse no.13.
4. The word Fateela is used 3 times, 2times in (Surah) Nisa Verses 49 and 77and V. 71 in (Surah) Isra.
5. Rutab in Chapter 19 (Surah) Maryam verse no 25.
6. The word Nava has been applied to the date- stone by Abdullah Yusuf Ali, Surah An 'am v, 45.
7. Similarly al-Urjoon means the lower part of the raceme of Dates which becomes dry and takes the shape of a sickle. Hence it has been compared with the appearance of the new Moon in Verse 39 of Surah XXXVI Yasin.

8. The words Habl and Dusur have also been translated by Yusuf Ali (Note 5138) as palm-fibre and are mentioned in Surah CXI (Lahab, V,5) and Surah LIV(Qamar,V.13) respectively.

SURAH Al-Hashr 59:5

مَا قَطَعْتُم مِّن لِّينَةٍ أَوْ تَرَكْتُمُوهَا قَآئِمَةً عَلَىٰٓ أُصُولِهَا فَبِإِذْنِ ٱللَّهِ وَلِيُخْزِىَ
ٱلْفَـٰسِقِينَ ﴿٥﴾

[5]Whatever you have cut down of [their] palm trees or left standing on their trunks - it was by permission of Allah and so He would disgrace the defiantly disobedient.

SURAH An-Nisa, 4: 53

أَمْ لَهُمْ نَصِيبٌ مِّنَ ٱلْمُلْكِ فَإِذًا لَّا يُؤْتُونَ ٱلنَّاسَ نَقِيرًا ﴿٥٣﴾

[53]*Or have they a share of dominion? Then [if that were so], they would not give the people [even as much as] the speck on a date seed.*

وَمَن يَعْمَلْ مِنَ ٱلصَّـٰلِحَـٰتِ مِن ذَكَرٍ أَوْ أُنثَىٰ وَهُوَ مُؤْمِنٌ
فَأُو۟لَـٰٓئِكَ يَدْخُلُونَ ٱلْجَنَّةَ وَلَا يُظْلَمُونَ نَقِيرًا ﴿١٢٤﴾

[124] *And whose will do some good deeds, may be a male or female and be a Muslim, then they shall*
enter Paradise, and they shall not be wronged a bit.

يُولِجُ ٱلَّيْلَ فِى ٱلنَّهَارِ وَيُولِجُ ٱلنَّهَارَ فِى ٱلَّيْلِ وَسَخَّرَ ٱلشَّمْسَ وَٱلْقَمَرَ كُلٌّ
يَجْرِى لِأَجَلٍ مُّسَمًّى ۚ ذَٰلِكُمُ ٱللَّهُ رَبُّكُمْ لَهُ ٱلْمُلْكُ ۚ وَٱلَّذِينَ تَدْعُونَ
مِن دُونِهِۦ مَا يَمْلِكُونَ مِن قِطْمِيرٍ ﴿١٣﴾

[13]*He brings the night into the day and brings the day into the night and He has engaged the sun and the moon into services; every one run up to an appointed time. This is Allah your Lord, His is the kingdom. And those whom you worship beside Him, own not even the husk of a date palm.*

SURAH Al-An'am 6: 95

۞ إِنَّ ٱللَّهَ فَالِقُ ٱلْحَبِّ وَٱلنَّوَىٰ ۖ يُخْرِجُ ٱلْحَىَّ مِنَ ٱلْمَيِّتِ وَمُخْرِجُ ٱلْمَيِّتِ
مِنَ ٱلْحَىِّ ۚ ذَٰلِكُمُ ٱللَّهُ ۖ فَأَنَّىٰ تُؤْفَكُونَ ﴿٩٥﴾

[95]*Undoubtedly! Allah is the Cleaver of seed-grain and date stone. He brings forth the living from the dead and is the Bringer forth of the dead from the living. This is Allah! Where are you going back?*

SURAH An-Nisa 4:49

أَلَمْ تَرَ إِلَى ٱلَّذِينَ يُزَكُّونَ أَنفُسَهُم ۚ بَلِ ٱللَّهُ يُزَكِّى مَن يَشَآءُ وَلَا يُظْلَمُونَ
فَتِيلًا ﴿٤٩﴾

[49]*Have you not observed those who declare themselves to be pure? Nay, it is Allah who purifies whomsoever He pleases, and they shall not be wronged a single date-thread.*


SURAH An-Nisa 4:77

أَلَمْ تَرَ إِلَى ٱلَّذِينَ قِيلَ لَهُمْ كُفُّوٓاْ أَيْدِيَكُمْ وَأَقِيمُواْ ٱلصَّلَوٰةَ
وَءَاتُواْ ٱلزَّكَوٰةَ فَلَمَّا كُتِبَ عَلَيْهِمُ ٱلْقِتَالُ إِذَا فَرِيقٌ مِّنْهُمْ يَخْشَوْنَ ٱلنَّاسَ
كَخَشْيَةِ ٱللَّهِ أَوْ أَشَدَّ خَشْيَةً ۚ وَقَالُواْ رَبَّنَا لِمَ كَتَبْتَ عَلَيْنَا ٱلْقِتَالَ لَوْلَآ
أَخَّرْتَنَآ إِلَىٰٓ أَجَلٍ قَرِيبٍ ۗ قُلْ مَتَٰعُ ٱلدُّنْيَا قَلِيلٌ وَٱلْءَاخِرَةُ خَيْرٌ لِّمَنِ ٱتَّقَىٰ
وَلَا تُظْلَمُونَ فَتِيلًا ﴿٧٧﴾

[77] *Have you not observed those to whom it was said 'restrain your hands, and establish prayer and pay the specified charity (Zakat), then when fighting was made obligatory on them, then some of them began to fear people as they should fear Allah or even more than that, And said they 'O Our Lord! Why have You prescribed fighting on us, You, would have allowed us to live for a short period'. Say You 'the enjoyment of the world is little and the Hereafter is better for the who fear Allah, and injustice will not be done to you, [even] as much as a thread [inside a date seed].*

SURAH Israel 17:71

يَوْمَ نَدْعُواْ كُلَّ أُنَاسٍ بِإِمَٰمِهِمْ ۖ فَمَنْ أُوتِيَ كِتَٰبَهُۥ بِيَمِينِهِۦ فَأُوْلَٰٓئِكَ
يَقْرَءُونَ كِتَٰبَهُمْ وَلَا يُظْلَمُونَ فَتِيلًا ﴿٧١﴾

[71]*The day when We shall call every people. With their leaders, then whosoever is given his record (deeds) in his right hand, they shall read their records and their rights" shall not be suppressed [even]as much as a single thread.*

Prophetic References:

- Hazrat Abdullah bin Umar (Radiyallahu Anhuma) narrated that The Rasulullah (Sallallahu Alayhi Wasallam) said, "There is a tree among the trees which is similar to a Muslim (in goodness). Its leaves do not fall. What is that tree?
  The Prophet (Sallallahu Alayhi Wasallam) himself said, “that is the date palm tree" (Bukhari, 1998; Muslim,1998).
- He told his followers to," Treat your aunt with honor: the palm tree and the dried grape"(Chaghhayni,1962).He also said ," The palm tree and the pomegranate were created with what was left over from the dust of Adam"(Chaghhayni,1962).
- According to Prophet (SAW)," The best of all your dates is that of al-barni, which drives out disease"(Sayuti,1994).
- Imam Ali ibn Abi Talib said," The best of dates are known as al-barni"(Majlisi, 1956).
- The Messenger of Allah encouraged the consumption of dated as a staple.
  According to both Bukhari and Ahmad, dates and water were the basis of the Prophet's diet (Nasai, 1964-65; Chaghhayni, 1962).
- He said," The date is the best breakfast for a believer"(Chaghhayni, 1962).
- He used to eat melon with fresh dates, saying that “one drives out heat, the other, cold"((Trimidhi, 1937).
- It is also reported that he said, “When dates are in season, congratulate me: when they are out of season, sympathize." (Chaghhayni, 1962).
- He used to break his fast with fresh dates and, if there were none, with dried dates (tamarat) (Ibn Hanbal, 1993).
- The Prophet also used to rub dates in the months of newborn babies (Bukhari, 1998).
- He said,"He who finds a date, let him break his fast with it. And he who finds no date, let him break his fast with water. For verily, that is purity"(Chaghhayni, 1962).
- The Messenger of Allah recommended dates for a host of medical reasons. He said,” Dates are a fruit which seed came from Paradise. They are an antidote of poison, a rich source for increasing semen, and drinking the water of soaked Madinah dates breaks the spell of witchcraft"(Nasai, 1964-65).
- He recommended people to "Eat fresh dates(balah), with dried dates(tamr),for when Satan regards, the son of Adam eating these two, he says : the son of Adam remained until he has eaten the new with the old"(Ibn Majah,1994).
- The Messenger of Allah recommended the consumption of dates by pregnant women, saying," your women –folk should eat dates, for whoever makes dates their food will produce children with ease "(Suyuti,1994).
- He stated," Fresh dates are the best food for a woman after she gives birth" (Ibn Habib, 1992).
- The Prophet is also reported to have said,"If one of your women has given birth to a child, see that the first thing that she eats fresh dates[rutb].Verily, Maryam[Mary] did not eat anything better than these when she gave birth to Isa[Jesus] (Chaghhayni,1962).
- Prophet Muhammed (Peace Be Upon Him) said that the best assets is date palm, dates cure several disorders, and he suggested Muslims to eat the date and have a tendency the date palm [Zaid A].

- The importance of dates has been documented in the Qur'an in Surah Maryam. One significant role of dates comes as when Mary gave birth to the Prophet Jesus (may peace be upon Him) under a palm tree, she heard a voice telling her: "Shake the trunk of the palm tree towards thee: it will drop fresh, ripe dates upon thee. Eat, then, and drink, and let thine eye be gladdened!" (Qur'an 19: 25-26). Aiwa is a types of dates, cultivated only in Saudi Arabia/Al-Madinah Al-Munawara and have significant value in diseases cure. The health benefit of Ajwa dates has been documented in hadith as Saud (R.A) narrated that I heard Allah's Apostle saying, "If Somebody takes seven Ajwa dates in the morning, neither magic nor poison will hurt him that day [Al-Bukhari, 1976]".

The fruits of dates has important place in religion

In Islam dates fruits are used to break the day long fast during the holy month of Ramadan [Al-Shahib, 2003]. This has been registered in the g. book of world records as the largest field of harvest in which there are 200,000 date trees (Fig.1). This is considered as the largest garden on the face of the earth.(This complete garden is devoted for providing the iftar in the Harams of Makkah and Madina during the month of Ramadhan).

The development cycle of the date fruit is divided into five (5) distinct stages:

1) Habakuk - development of a small white fruit after a week of pollination
2) Kimri - At about 5-17 weeks there is a visible change of fruit color and size. The fruit is small and green and slowly turns yellow and red.
3) Khalal - In 18 -25 weeks, the fruit grows longer and turns either fully yellow or red. The fruits are quite hard but can be consumed.
4) Rutab - the fruit ripens in another 5-7 weeks, turns brown in color and getting ready to be harvested
5) Tamr - 3 weeks post the rutab stages, the ripened fruit is harvested.
   There are more than two hundred varieties [Amer, 1994] of dates available worldwide. It is the main crop in Egypt, Saudi Arabia, and Middle Eastern countries. It is thought that the native origin of dates is around the Persian Gulf, and has been cultivated from Mesopotamia to prehistoric Egypt as early as 4000 BCE. Due to the old historical prospective of date, the exact date of origin is very difficult to identify [Chao CT, 2007]. Most likely it originated 4000 BC from the ancient Mesopotamia area (southern Iraq) or western India [Wrigley G., 1995].

Latest Scientific studies on dates

Dates aren't exactly a nutritional powerhouse when compared to some other foods like kiwi or sesame seeds, but the fruit does still offer numerous health benefits along with great taste. Here are some health benefits of dates:

1. Promoting Digestive Health, Relieving Constipation - fiber found in dates help to clean out the gastrointestinal system, allowing the colon to work at greater levels of efficiency. Some other benefits relating to fiber and colon health are reduced risks of colitis, colon cancer, and hemorrhoids
2. Boosting Heart Health – In addition to promoting colon health, fiber is also known to boost heart health.

3. Anti-Inflammatory – Dates are rich in magnesium – a mineral known for its anti-inflammatory benefits.
4. Reduced Blood Pressure – Magnesium has been shown to help lower blood pressure – and again, dates are full of the mineral.
5. Dates are low in fat and high in carbohydrates and other elements. Research studies have reported that dates are rich in macro elements mainly potassium, phosphorous, calcium, chlorine and magnesium and have appreciable quantities of microelements essentially iron, manganese, copper and zinc.
6. The fruit have antioxidant flavanoids such as beta-carotene, lutein, zea-xanthin and tannins. These antioxidants possess anti-inflammatory, anti-hemorrhagic and anti-cancer properties.

The UAE has been at the forefront of those countries that granted special attention to the agricultural sector and date palm. It has constructively contributed to upgrading this sector through the preservation and development of resources in support of world food security through a variety of purposeful initiatives and successful events, which achieved a great success for the Arab and International community.

This special attention is clearly evident in the continued expansion in agricultural resources and investments, in the fast growth in the number of palm trees (Fig. 2), in the continued increase in the size and variety of date projects, in the extensive use of modern technologies, and in the important initiatives undertaken in the areas of manufacturing and marketing of date fruits.

Under the leadership of His Highness, The President Sheikh Khalifa Bin Zayed Al-Nahayan, there are continual efforts to increase agricultural productivity, to make better use of available resources, and to produce an agricultural leap that is changing the face of the UAE's desert.

The founder of UAE attached a great importance to agricultural development in general, and to the date palm in particular. This attention is clearly evident in the fast growth in the number of palm trees in the continued increase in the size and variety of date products, in the extensive use of modern technologies, and in the important initiatives undertaken in the areas of manufacturing and marketing of date fruit. The number of the date palms is about 100 million worldwide, of which 62 million palms can be found in the Arab world.(2).

The UAE Government, within the framework of its development plans, has placed the establishment of a date production industry as one of its important priorities. Under the leadership of His Highness, The President, Sheikh Khalifa Bin Zayed Al Nahyan [Almighty Allah (SWT) protects Him], there have been continual efforts to increase agricultural productivity and to make better use of all available resources.

Good Agricultural Practices (GAP) for Date Palm Production

- Selection of Medicinal Plants
- Documentation
- Seeds and Propagation Material
- Cultivation

Cultivation of Date Palm requires intensive care and management

- Site selection
- Ecological Environment
- Soil
- Irrigation and Drainage
- Plant maintenance and protection
- Harvest
- Personnel

Site requirements

Temperature

Date palm requires an arid climatic (hot & dry) with a temperature between 25^0C to 320 and a sufficient water supply. Daily maximum temperature below 9^0 C and a minimum temperature below 0^0C are growth- inhibiting and temperature around 7 ^{0} C cause damage. As a precondition for flowering date palms need temperature over 18^0C (in the shade) and for fruit setting temperatures above 25^0 C.

Good Field Collection Practices (GFCP) for Date Palm

Technical planning:

- Prior to field collection the population density & geographic distribution of the target medicinal plant species must be identified.
- Topographical map of the collecting site should be prepared.
- Field collection team and their responsibilities should be clearly identified.
    1. Permission & Collection Permit: must be obtained prior.
    2. Selection of Medicinal Plants for Collection: Collected medicinal plants should be the same as those specified in national pharmacopoeia.
    3. Collecting Techniques & Procedures:
       Ecologically non-destructive means of collection should be employed.

Technical Aspects for GACP & GFCP

- Post-harvest processing
- Bulk packaging and labeling
- Storage and transportation
- Equipment

Control practices using chemicals is one of the quick solution of pest problem. But pesticides have a hazardous effect on environment and side effects on the consumers of crops, whereas, Bio pesticides are inherently less harmful than conventional pesticides and are designed to affect only one specific pest or, in some cases, a few target organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects, and mammals. Bio pesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides. When used as a component of Integrated Pest Management (IPM) programs, bio pesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. Bio pesticides play an important role in providing pest management tools in areas where pesticide resistance and environmental concerns limit the use of chemical pesticide products. Keeping in view the above fact we worked on Bio pesticide Management Program.

In continuation of our earlier work (1), the present study is a step forward for carrying out the Use of Bio-Pesticide for Sustainable Date Palm Production to identify and evaluate suitable and new plant extracts with eco-friendly activities against endoparasitic Larval Red Palm Weevil, *Rhynchophorus ferruginous* Oliver found in Date Palm tree (*Phoenix dactylifera* L). Also deals with morphoanatomical; pharmacognostic studies; physicochemical protocols; phytochemical screening using chromatographic fingerprinting; spectral techniques and the quantitative analysis of inorganic elements present therein the date palm and plant extracts used as bio pesticides for sustainable date palm production.

The following studies were carried out: Preparation of the sample and different plant extracts ; acute toxicity studies ; serum urea and uric acid analysis ; macroscopy; microscopy ; chemical constituents ; qualitative tests for the presence of following classes of organic constituents: flavanoids; steroids ; carbohydrates ;alkaloids; tannins; phenolics ; amino acids; starch saponins using thin layer chromatographic studies; HPLC studies: IR Spectra: UV / VIS spectrum; inorganic constituents. Animals (Albino mice) treated daily with palm sap for 14 days did not show any change in urea or uric acid. No toxic signs were observed during any of the treatment period. Quantitative and chromatographic results showed the presence of many amino acids (e.g. glutamic acid, alanine, valine, isoleucine), vitamins (e.g. B_1, B_2 and vitamin C); steroids (e.g. cholesterol, Stigmasterol, lupeol, β-sitoserol and campsterol), besides presence of tannins & carbohydrates. The major inorganic constituents present are calcium, magnesium, a high percentage of potassium, besides aluminium, iron, phosphorus and small amounts of zinc, nickel & manganese.

Re-trials were conducted 'invitro' in the lab nine petridishes were taken and in each of the petridishes 4 large uniformly sized larval Red Palm Weevil were taken along with some plant fibers. Each petridish was numbered starting from 1 to 9. Each petridish was poured with mixture of plant extract and water in ratio of 3 gm of the plant extract to 50 ml water. The larvae were allowed to be in the petridishes filled with trial plant extract water for about 10 minutes. Larvae were allowed to be in the mixture in the petridishes for about 10 minutes. Then the larvae were removed and were spread on bloating papers separately to wipe out excess plant extract / insect water from their bodies. Then they were observed for the effect of the test so on the larval mortality and the follow results were observed.).

Bio pesticides of plant origin could be the key to the future sustainable date palm production. The above two plant extracts have been found very effective to kill the aforesaid pest insects: *Azadirachta indica* (Neem) Seed and seed kernel and *Annona sqamosa*.

As per our previous results, the botanical extracts of two plants have a promising results in the larval Red Palm Weevil i.e. the in vitro studies in the laboratory showed 100% mortality of these larvae. In vivo studies on infected live tree were initiated as reconfirmation. Application of these bio pesticides will not affect the taste and behavior of the date palm fruits as it is affected by the application of organophosphorus insecticide/ pesticides earlier. This programme has not only of national significance but if proved successful, would be applicable worldwide for the sustainable growth of date palm production. Further progress of experiments will be dealt in detail.

REFERENCES

A. Zaid, P.F. De Wet, M. Djerbi and A. othabi and the references therein. Chapter XII. Disease and Pest of Date Palm.

Zohary D, Hopf M. Domestication of Plants in the Old World. 2nd edition. Oxford: Clarendon; 1993. Date palm Phoenix dactylifera.

δάκτυλος. Liddell, Henry George; Scott, Robert; A Greek–English Lexicon at the Perseus Project.

fĕro. Charlton T. Lewis and Charles Short. A Latin Dictionary on Perseus Project.

Sulieman A, Elhafise I, Abdulrahim A. Comparative study on five Sudanese date (Phoenix dactylifera L.) fruit cultivars. Food Nut Sci. 2012; 3:1245–1251.

Proceedings from "The First International Conference on Date Palms" UAE University, Faculty of Agricultural Sciences, Dept. of Plant Production. Al Ain 1998.

Prof Hamad Abdel-reheem Ead "The Date Palm in Ancient History". (Prof Hamad is Professor of Chemistry, Faculty of Science, University of Cairo and Director of the Science Heritage Centre.

Yaarub Al Yahya "Biotechnology and Date Palm Development" (Yaarub is from Wye College, University of London).

Rashid Al Yahyai " The Traditional Date Palm Cultivation in Oman" (Rashid is at Cornell University? department.)

W.H. Barreveld "Date Palm Products", FAO Rome, 1993.

Beech M. and Shepherd E. "Archaeobotanical evidence for early Date consumption on Dalma Island, United Arab Emirates", Antiquity 75 (2001): 83-9

Ali Ahmad Al Shahri "The Language of Aad" (Essentially this book is the story of the Sharha, first Arabs and their language, Sharhi) - privately published in 2000- difficult to find.

M.Kamil 'Use of Bio-Pesticide-New Dimension and Challenges for Sustainable Date Palm Production, Acta Horticulture, No.882,p95- 102, Dec'2010.

A. Zaid, P.F. De Wet, M. Djerbi and A. othabi and the references therein. Chapter XII. Disease and Pest of Date Palm.

Mohammed A. Fayyad, Ali Z. Abdalqader, Amged A. Fadel, Eman A. Muhammed, Ala A.

Manea, Muhamud A. Jaffer, Azhar A. Fadel Formulation of biopesticide from Beauveria bassiana as part of biological control of date palmstem borer (Jebusaea hammershmidti) AAB Bioflux, 2013, Volume 5, Issue 2.84-90

M. Kamil ,Use of Bio-Pesticide - NEW Dimension & Challenges for Sustainable Date Palm Production, Fourth International Conference on date palm, Abu Dhabi, United Arab Emirates;15-17 March,2010.

Rieger, M. (2006) Introduction to Fruit Crops. Food Products Press, Binghamton, US.

2-UC Davis Department of Plant Sciences (October, 2010)

http://www.plantsciences.ucdavis.edu/GEPTS/pb143/CROP/DATE/date.htm

Zaid A, De Wet PF. Origin, geographical distribution and nutritional values of date palm. Date Production Support Programme FAO

Al-Bukhari MI. In: The collection of authentic sayings of Prophet Mohammad (peace be upon him), division 71 on medicine. 2nd edition. Al-Bukhari S, editor. Ankara, Turkey: Hilal Yayinlari; 1976.

Al-Shahib W, Marshall RJ. The fruit of the date palm: Its possible use as the best food for the future. Int J Food Sci Nutr. 2003; 54:247–259. [PubMed]

Amer WM. Ph.D. Thesis. Giza: Cairo University; 1994. Taxonomic and Documentary Study of Food Plants in Ancient Egypt.

Chao CT, Krueger RR. The date palm (Phoenix dactylifera L.): Overview of biology, uses, and cultivation. Hortsci. 2007; 42:1077–1082.

Wrigley G. Date palm. In: Smartt J, Simmonds NW, editors. Evolution of crop plants. 2nd edition. Essex, UK: Longman Group; 1995. pp. 399–403.

http://naturalsociety.com/health-benefits-of-dates-7-reasons-eat-date-fruit/

http://www.organicfacts.net/health-benefits/fruit/health-benefits-of-dates.html
Bukhari M. *Sahih al-Bukhari.* Al-Riyyad: Bayt al-Afkar,1998; Muslim.Jami al-sahih.al Al-Riyyad: Bayt al-Afkar,1998;Ibn Majah M *sunnan*. MT Ansari.Lahore:Kazi
publication,1994;Tirmidhi M. *al-jami al-sahih.*al Qahirah: Mustafa al-Babi al-Halabi[1937].
Chaghhayni M. *Tibb al-Nabi.* Trans.C Elgood. *Osiris* 1962; 14:189.
Chaghhayni M. *Tibb al-Nabi*.Trans. C Elgood. *Osiris* 1962; 14:190; 191.
Majlisi M. *Bihar al-anwar.* Tihran: Javed al-Alavi, 1956.
Suyuti J. As-suyuti's Medicine of the Prophet.Ed.A Thomson. London::Ta-Ha
Publishers,1994:63;Chaghhayni M. *Tibb al-Nabawi.* Trans. C Elgood.Osiris 1962;14:190; Chishti SHM. *The book of Sufi* Healing.Rochester,Vermont: Inner Traditions International,1991:62.
Suyuti J. As-suyuti's Medicine of the Prophet.Ed.A Thomson. London::Ta-Ha Publishers,1994:63; 109.
Suyuti J.Jami al-ahadith.Bayrut Dar al-Fikr,1994; Ibn Qayyim al-Jawziyyah M. *al- Tibb al-nabawi.*Bayrut: Dar al-Kitab,1985.
Suyuti J.Jami al-ahadith.Bayrut Dar al-Fikr,1994;Muttaqi A. *Kanz al-ummal* .2nd ed. Hyderabad: al-Dikin Dairat al-ma'arif al-'Uthmaniyah,1945-75;Isbahani AN al-*Mawsuat al-tibb-al nabawi.* Ed.MKD al-Turki.Bayrut,Lubnan:Dar Ibn Hazm,2006.
Suyuti J.Jami al-ahadith.Bayrut: Dar al-Fikr, 1994; Muttaqi A.Kanz al-ummal. 2nd ed. Hyderabad:Dairat al-maarif al-Uthmaniyyah,1945-75.

Figures

Fig. 1: Garden of Al-Rajhi at Qaseem in Riyadh –Saudi Arabia.

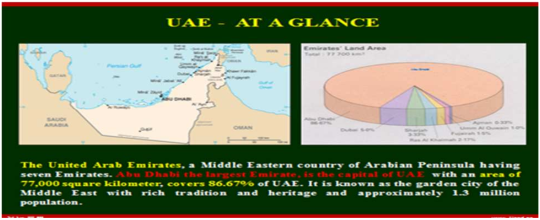

Fig. 2:

MiSeq analysis reveals high fungal diversity and the presence of new fungal pathogens of date palms

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Abstract

Date palm is the most important crop in the Sultanate of Oman, with a total production of 328,000 tonnes. Date palm faces several challenges, with wilt diseases being of an increasing threat. A survey in different farms in Oman showed that wilt is widespread in several date palm cultivars and regions. Isolations from diseased trees followed by identification of the isolated fungi using sequences of the Internal transcribed spacer region of the ribosomal RNA (ITS rRNA) showed that several fungal species are associated with the disease, including species belonging to *Fusarium*, *Lasiodiplodia* and others. In addition, MiSeq analysis was conducted on DNA extracted directly from date palm roots, trunk and leaves, as well as from the rhizosphere of date palms. The analysis showed the presence of a high level of fungal taxa in date palm soil, mostly dominated by saprophytic fungi and some potential biocontrol agents, with the minority of fungi being pathogenic on date palms. Soils from farms suffering from wilt symptoms were found to have higher levels of fungal pathogens, suggesting that the imbalance in the soil microflora could lead to fungal pathogens causing diseases in date palms. Variations were found in the fungal taxa among date palm roots, trunk and leaves, suggesting that each date palm part is affected by different fungal pathogens. This study reports new fungal pathogens on date palms. It also suggests that the rhizosphere of date palms is usually dominated by saprophytic and antagonistic fungi that help minimize the impact of diseases on date palms.

Effect of plant extract Ruta graveolens against the date scale, Parlatoria blanchardi Targ., (Homoptera, Diaspididae) at Biskra oasis, Algeria

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Abstract

The date scale Parlatoria blanchardi (Targ.1868), (Homoptera, Diaspididae) is one of the most devastating pests on date palm. The damages caused at Biskra oasis are considerable. To minimize the side effect of chemical use against date scale, a survey was conducted at Oumache, Oasis of Biskra, by applying plant extract of common rue, Ruta graveolens (Rutaceae). Extracts were sprayed on the first, second and sixth day. Three different extracts were used; seed extract, oil extract from the seed and extract from dry leaves, with three concentrations (0.25, 0.5, 1.0 ml/ml). Extracts were tested on different larval stages and adults under laboratory or field conditions, during the autumn and winter period of the year 2015. Results showed that the mortality level increased with increasing extract concentration, especially on the second and third larval stages. The high cumulative level of larvae mortality was found 72 hours after oil extract treatment, with 83% larvae mortality under laboratory conditions and 70% larvae mortality in the fields.

Keywords: palm-date – scale-Oasis- Biskra- plant extracts.

Phylogenetic and pathogenic characterisation of *Mauginiella scaettae* as the causal agent of date palm (*Phoenix dactylifera* L.) inflorescence rot south east of Algeria

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Abstract

Inflorescence rot is a devastating disease of date palm (*Phoenix dactylifera L.*) but has not been extensively characterised. *Mauginiella scaettae* Cav. 1925 (Pleosporales; Ascomycota), causal agent of this disease, was isolated from infected inflorescences of date palm both males and females sampled from different oases of the south east of Algeria: Ouargla, Tougourt, Hadjira, Oued and Biskra. The isolated GA strain of *Mauginiella scaettae* was used to infect healthy mal inflorescence of date palms to satisfy Koch's postulates. Phylogenetic reconstruction using the rRNA locus internal transcribed spacer (ITS) region revealed a close relationship between the Algerian strains and the only previously isolated *Mauginiella scaettae* from Spain. More widely, *Mauginiella scaettae* was confirmed fall within family Phaeosphaeriaceae closely related to genus *Phaeosphaeria* which includes many pathogenic species. Fourier Transform Infrared spectroscopy (FTIR) metabolite fingerprinting was employed to discriminate fungal strains between those that originated from male and female inflorescences. Scanning electron microscopy (SEM) characterisation of the infection processes for *Mauginiella scaettae* in both male and female inflorescences suggested a preference for stomatal entry. Development of a heterologous pathosystem based on *Arabidopsis thaliana* was used to confirm the virulence of the *Mauginiella scaettae* strains, host penetration via stomata and a final necrotrophic lifestyle. Our characterisation provides new insights into this inflorescence rot to allow better detection and management of the disease.

Key words: Date palm, Southeast Algeria, Inflorescence rot, *Mauginiella scaettae*, ITS, FTIR, *Arabidopsis thaliana*, SEM.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious tree species which is one of the most important crops in North Africa. Date production in Algeria varies annually but has surged from 205,907 MT in 1990 to 755, 000 MT tonnes in 2011 (Benziouche, 2012), with 7% of the total world date production originating from Algeria in 2008 (FAOSTAT, 2009). However, the date palm sector in Algeria is being compromised by burial of palm groves due sand accretion and the effects of severe drought (Touzi, 2007). Losses due to pests and pathogens are also significant (Carpenter and Elmer, 1978; Djerbi, 1982; Ploetz et al., 2003; Abdullah et al., 2005) with serious damage linked to Bayoud disease caused by *Fusarium oxysporum* f.sp. *albedinis* (Touzi, 2007).

One of the major diseases of date palm is inflorescence rot (known as Khamedj disease in North Africa) which in severe cases can destroy around 50-80% of the date palm inflorescences (El-Baker, 1962) and is thus a major factor limiting yield. Disease symptoms appear on young spathes, especially inward facing side when the spathe is still hidden in the leaf bases (Carpenter and Elmer, 1978). Inflorescence development occurs during the hot and humid season (March-May), especially following periods of heavy rain. Disease transmission occurs through the infection of male inflorescences during the pollination period (Ploetz et al., 2003; Abdullah et al., 2005). Al-Ani et al. (1971) found that the disease persisted on the trees, and that infected trees formed diseased inflorescences for several years. *M. scaettae* is frequently isolated infected inflorescences of date palm and though it is suspected to be the causal agent.

Colonies of *M. scaettae* are fast-growing on agar media (radial growth rate of <2 cm after growth for 10 d at 25°C on PDA (Potato Dextrose Agar). Colonies are white and sporulate abundantly, leading to a powdery appearance. The mycelium is composed of branched hyaline septate hyphae with immersed hyphae 1.5-2.5 μm wide and aerial hyphae 3-4 μm wide. Arthroconidia are produced by segmentation of the aerial hyphae, and may be unicellular or septate, up to 35 μm in length (Cavara, 1925a ; Arx et al., 1981; Abdullah et al., 2005).

This disease and its presumed causal fungus were first reported by Cavara (1925 a,b) in Cyrenaica, Libya and more widely (Chabrolin, 1928; Maire and Werner, 1937; Munier,1952; Munier, 1955; Calcat, 1959; Rattan and AL-Dboon, 1980), including Egypt (Michael and Sabet, 1970), the Arabian Peninsula (Abu Yaman and Abu Blam, 1971; Djerbi, 1982), Iraq (Allison, 1952; Hussain, 1958; Al-Ani et al., 1971) and Southern Spain (Abdullah et al., 2005). However, other fungi including *Fusarium oxysporum* Schldl. emend. Snyder and Hansen, *Fusarium moniliforme* Sheld. Aggregate, *Fusarium solani* (Mart.) Appel and Wollenw. aggregate Snyder and Hansen, *Thielaviopsis paradoxa* (Dade) C. Moreau. and *Trichothecium roseum* (Pers.) Link, are also reported to be associated with diseased date palm inflorescences (Brown and Butler, 1938; El-Behadili et al., 1977; Rattan and Al-Dboon, 1980).

Al-Ani et al., (1971) reported that date palm cultivars differed in their reactions to the disease. Out of the five most common commercial cultivars in Iraq, 'Hillawy' was highly resistant, while 'Khadhrawy' was highly susceptible (Abdullah et al., 2005). Within an Algerian context, one of the most common date palm cultivar is Deglet Nour (~45%) (Bouguedoura et al., 2008). which displays greater resistance to inflorescence rot than cultuvar Ghars. However, cultivars Tafezouine, Hamraia and Takermost were resistant (Teisseire, 1961). Clearly, wholesale replacement of the date palm population with more resistance cannot be considered a short-term option. Instead, careful monitoring of the dispersal patterns of the most virulent causal agent, coupled with robust phytosanitary measures and effective fungicide use is required.

The aim of this study was to identify the isolates from infected or diseased date palm inflorescences and obtain additional evidence that *M. scaettae* is the causal agent. The latter involved using *Arabidopsis thaliana* as a heterologous host for *M. scaettae*. We also characterised the infections strategies of *M. scaettae* in homologous and heterologous hosts which seemed to involve an initial biotrophic prior to a necrotrophic phase. Based on these observations, we suggest inflorescence rot can be better detected and controlled based on such as PCR-based detection of early infection.

MATERIALS AND METHODS

i) Purification of fungal strains from disease date palm inflorescence

Sampling of diseased male and female date palm inflorescences was undertaken in March-May 2012 in southeast of Algerian oases up to 250 km apart: Ouargla, Touggourt, Hadjira, Oued, Biskra, Ghardia (Supplementary Fig. 1). The annual temperature range is 14-26°C and monthly rainfall is 2-7 mm (Algerian meteorological office, 2008 - http://www.meteo.dz/). These areas were surveyed following previous reports of inflorescence rot.

The fungus was isolated from the spathe, flowers and strands of the infected inflorescence by sterilizing the edges of the affected area with 90% ethanol or 10% sodium hypochlorite. The parts were then placed in a sterilized Petri dishes containing 10% sodium hypochlorite solution (10 min) and washed repeatedly in sterilized water before drying between sheets of sterile blotting paper. Samples were then transferred to Petri dishes containing PDA (Potato Dextrose Agar, Oxoid, UK) and incubated on 22°C in dark. Plates were examined daily for growth and pure cultures were obtained by repeated isolation of any emergent fungal mycelia.

Radial growth rates were measured for up to 10 days (PDA, 22°C in the dark). For micro-morphological observations, mycelial samples were obtained from cultures grown on PDA, The terminology used for description of the mycelium and arthroconidia follows that used by (Chabrolin, 1930; von Arx and al., 1982; Abdullah et al., 2005). Images were captured using a Nikon Coolpix 995 digital camera installed on an Olympus BX50 light microscope.

ii) Pathogenicity test

Artificial inoculations have not been conducted on natural host. Arthroconidia from strain GA (Table 1) grown on PDA) were used for pathogenicity assays, with a suspension of arthroconidia (10^5 spores.ml^{-1}) prepared in sterile water. Healthy (male) spathes were surface-sterilized with 95% ethanol, and the upper external surface of the spathe was removed so that the flowers and strands were exposed to the atmosphere. These were inoculated by spraying with 20 ml of the arthroconidia suspension per inflorescence. Inoculated spathes were placed in sterile plastic bags and incubated at 22^0C in the dark. Spathes were examined for symptoms after 1 week of incubation.

iii) Re-isolation of the pathogen

To satisfy Koch's Postulates, parts from inoculated inflorescence parts (Spathe, flowers and stands) exhibiting disease symptoms were surface-sterilized (5min) with 5% sodium hypochlorite, washed three times with sterile distilled water and plated on PDA. Plates were incubated at 22°C and the subsequent growth of the pathogen was recorded.

iv) Genomic DNA extraction and PCR amplification and DNA sequencing

Cultures were grown on PDA (three replicates per strain) and mycelium scraped from the agar surface after ten days of growth, frozen and ground with liquid nitrogen in microcentrifuge tubes. Genomic DNA was extracted and isolated using a QIAGEN DNeasy Plant mini kit (Qiagen, Manchester, UK) following the manufacturer's protocol.

The universal primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') primers were used to amplify amplification of the

ITS region (White et al., 1990; Gardes and Bruns, 1993; Abdullah et al., 2005;Toju et al., 2012; Seifert, 2009; Kiss., 2012; Schoch et al., 2012). Amplification was conducted as follows : 4 µl of 10X Thermo buffer, 4 µl dNTP (0.2 mM final concentration), 3.2 µl $MgCl_2$ (2 mM), 0.1 µl (0.5U) *Taq* DNA polymerase, 2 µl of each primer (0.5 µM), 20 µl dH_2O, and 20 µl DNA template in a total volume of 40 µl. The polymerase chain reaction (PCR) was performed in a GenAmp 9700 thermal cycler (Mycycler, Bio-Rad, UK) using the following conditions: initial denaturation for 4 min at 94°C, 35 cycles of 50 s of denaturation at 94°C, 60 s of annealing at 56°C and 50 s of extension at 72°C; this was followed by final extension for 5 min at 72°C. The PCR products were separated on a 1.2% agarose gel, stained with ethidium bromide and viewed with ultraviolet light. PCR amplified fragments were purified using QIAquick PCR columns (Qiagen, Manchester, UK) .
Samples were sequenced in a 377 agilent sequencer (Fisher Scientific, UK). Sequences were submitted to the NCBI GenBank database (Table 1).

Phylogenetic Analyses

Sequence management was conducted within the Geneious v5.5.6 bioinformatics package (Drummond et al., 2011). Sequences were aligned using MAFFT (Katoh et al., 2002) using default setting and trimmed to remove flanking 18S/28S sequences. Phylogenetic reconstruction was conducted using PhyML (Maximum Likelihood)and the GTR (Generalised time reversible) substitution model with 1000 bootstrap replications (Guindon et al., 2010).

vi) Inoculation of *Mauginiella scaettae* on *Arabidopsis thaliana.*

A. thaliana Col-0 (Columbia (Col-0) ecotype, plants were cultivated as described by Johnson et al. (2003) in Levington Universal compost in trays with 24-compartment inserts. Plants were maintained in Conviron (Controlled Environments Ltd, http://www.conviron.com/) growth rooms at 24°C with a light intensity of 110 $\mu mol.m^{-2}.sec^{-1}$ and an 8 h photoperiod for 4 weeks. Aerial plant parts were inoculated at the fully expanded rosette stage at 5 weeks (stage 3.7 as defined by Boyes et al., 2001) as follows:

M. scaettae arthroconidia were harvested adding 1ml H_2O to the surface of 10 days old colonies and then rubbing the colony surface with a sterile glass spreader as described by Johnson et al., (2007) for preparation of Botrytis cinerea conidia. The initial arthroconidial suspension was diluted to 10^4 or 10^5 $spores.ml^{-1}$ in PDB (Potato Dextrose Broth; Formedium, UK)., Single leaves (leaf stage 7 or 8, as defined by Boyes et al., 2001) were inoculated with 5 µl of spore suspension pipetted onto the adaxial surface of the leaf. Controls were inoculated with PDB (Fig. 6).

vii) Fourier transform-infrared (FT-IR) spectroscopy

FT-IR reflectance spectra were obtained using a Bruker Vertex 70 spectrometer (Bruker Optics; http://www.brukeroptics.com/), as described in Gidman et al., (2003), but using a mercury–cadmium telluride (MCT) detector to measure sample reflectance. Data were analysed using MATLAB version 6.5 (MathWorks; http://www.mathworks.co.uk/). In-house algorithms were used to convert the resultant spectra into absorbance. The CO_2 peaks were replaced with a smooth trend using an in-house code and the spectra were normalised to total absorbance (Timmins et al., 1998).

PCA and PC-DFA were used as described in Allwood et al. (2006) and followed accepted Metabolomics Standards Initiative (MSI) standards (Goodacre et al., 2007). The PCA reduces the dimensionality of multivariate data whilst preserving most of the variance following which DFA is used to discriminate between groups on the basis of the retained PCs and the *a priori* knowledge of class structures within the datasets (MacFie et al., 1978; Windig et al., 1983). This process does not bias the analysis in any way.

viii) Staining and microscopy

For light microscopy infected leaves were stained with trypan blue or viewed under epifluorescence illumination after straining with aniline blue, as described in Routledge et al. (2004), using an Olympus BX50 microscope. Images were captured with a Nikon Coolpix 995 camera.

For scanning electron microscopy (SEM), freeze-substitution was carried out using a Leica EM AFS unit. Substitution fluids were pre-cooled to 193°K before the frozen tissue was transferred into them for substitution. Transfer was performed using suitably pre-cooled and insulated tools. Critical point drying of freeze-substituted samples was performed from 100% acetone at 283°K, in a Polaron E3000 unit, utilizing liquid carbon dioxide. Specimens were cooled to liquid nitrogen temperature and fractured, in the plane parallel or perpendicular to the tibial plateau, under vacuum using the steel blade in a modified Polaron freeze fracture unit. The specimens were then brought back to room temperature, whilst being kept under vacuum to prevent artefacts forming due to water condensing on the cold specimens, and mounted on specimen stubs. Specimen coating, in all cases, was carried out in a Polaron E5000 sputter coating unit with a cooled specimen holder, utilizing a platinum target and argon gas to provide approximately 10 nm coating. High resolution images were also obtained on Hitachi 4700 Scanning Electron Microscope.

RESULTS

i) Date plate inflorescence rot symptom on its natural host.

The disease is first seen in late winter or early spring as the spathes begin to emerge. On male date palm (Fig. 1 A,B) infected spathes first exhibit rot symptoms when they begin to emerge in early spring. Typically, these are seen as necrotic spots and patches on the spathe (Fig. 1 C). As the spathe matures and splits open, white fluffy mycelium can be seen covering the flowers, especially at the top of the spathe (Fig. 1D). Tissue necrosis is progressively observed inside the spathe and hyphae are also observed on the outside of the spathe (Fig. 1E). In female inflorescences, the flowers erupt rapidly from the spathe (Fig. 1F). Necrotic (rot) symptoms are observed from flowers emergence in late spring onwards, as pollination is in progress. Generally, symptoms begin as a white mycelial mass attacking the flowers (Fig. 1G). The spathe usually splits to reveal partial to near complete coverage of the flowers with white mycelium. As the infected flowers emerge, the areas previously covered white mycelium become necrotic- (Fig. 1H) killing the mature flowers (Fig. 1I). Rot symptoms are most severe on neglected palms in the marginal areas of oases, and waterlogged salty soil. Some of these palms develop symptoms every year and may represent reservoirs for annual cycles of infection.

ii) Culturing of fungi from inflorescence rot disease date palm

Explants from date palm inflorescence were obtained from trees exhibiting inflorescence rot, including trees of both sexes, ranging in age from 35-60 years (Table 1). Fungal colonies emerging from surface-sterilised infected (symptomatic) tissue explants were white (Fig. 2A) and similar to the growth seen on infected spathes (Fig. 1). Repeated subculture led to the purification of a series of strains from each spathe, all of which appeared to have an identical white appearance (Fig. 2B). Isolates were up to 5 cm diameter after 10 days of growth at 22°C on PDA. The underside of these colonies became light olive green with darker edges after prolonged culture. Representative strains we selected for each spathe which were designated according to their origins (Table 1).

Each strain was examined under the light microscope to reveal identical hyphal and arthroconidial morphologies (Fig. 2C). The mycelium was hyaline, branched and septate and produced 1 or 2 celled hyaline conidia as long chains. Arthroconidial dimensions were 12-60 x 8-12 μm. Immersed hyphae were 1.5-2.5 μm and aerial 3-4 μm wide. Arthroconidia were produced by segmentation of the aerial hyphae (Fig. 2C, h). This lead to the production of unicellular non-septate conidia (Fig. 2C a1) or multicellular septate arthroconidia (Fig. 2C a2, a3 and a4). These features were consistent with earlier descriptions of *M. scaettae* (Cavara, 1925a ; Chabrolin, 1930 ; van der Walt and Hopsu-Havu, 1976; von Arx and al., 1982; Abdullah et al., 2005).

iii) Pathogenicity test.

Parts of inoculated detached inflorescence (flowers, stands and spathes) were inoculated with spore suspensions of *M. scaettae* strain GA. These all developed clear inflorescence rot symptoms (Fig. 3A) after incubation for 7 days. Arthroconidia from artificially inoculated inflorescence were plated out on PDA and grew into colonies identical to those isolated from naturally infected tissues. (Fig. 3B)

iv) Genetic and Phylogenetic analyses of the derived ITS sequences.

Confirmation of the identity of the isolated fungal strains was obtained by amplification and bidirectional sequencing of the ITS region. The finalised sequences deposited in Gen Bank (KT587185-587194; Table 1). In all cases sequences were identical to *M. scaettae* (AY965895; from Spain) thereby confirming the identity of these isolates. *M. scaettae* was previously assigned to the ascomycetes family Phaeosphaeriaceae (Abdullah et al., 2005) but a recent phylogenetic revision of this family (Phookamsak et al., 2014) failed to confirm that *M. scaettae* belonged to this family. Phylogenetic reconstruction undertaken here similarly did not confirm its placement within this family and sequence analysis using additional loci would be necessary to place it unambiguously. using a range of sequences from other members of Phaeosphaeriaceae showed that *M. scaettae* sequences formed a distinct and well supported clade within this family. No sequence polymorphisms were observed within the ITS1 or ITS2 regions of the ten *M. scaettae* and all were identical to the single pre-existing GenBank sequence from Spain sequence from Spain (Abdullah et al., 2005).

Although the ITS sequence did not separate individual *M. scaettae* isolates, strain differences were revealed using FTIR metabolite fingerprinting. Fungal mycelium was cultured in PDB, centrifuged and spotted on to a silica plates for assessment using FTIR reflectance spectroscopy. The derived spectra were rich in information (Fig. 5A) which was assessed using chemometric methods. Investigating the spectra using Principal Component Analysis (PCA) failed to real any strain specific variation. As a result, Discriminant Function Analysis (DFA) a supervised approach were the algorithm was given *a priori* information of the classes of data linked to each strain. DFA based on 4 principal components (PC) explaining 96.5% of the total variation now were able suggest some differences between the stains (Fig.

5B). Interestingly, the strain exhibiting the greater difference to the others (across Discriminant Function 1) was OU3 as with the ITS sequence data (Fig. 4). Across DF2, two clusters of strains could be observed and these corresponded to strains originating from either male or female inflorescences (Fig. 5B).

v) Characterising the infection strategy of *Mauginiella scaettae*

In order to determine the infection strategy employed by *M scaettae* disease inflorescences were examined using SEM. The diseased spathes were covered with arthroconidia, identical to those form in pure cultures of *M. scaettae* (Fig. 6A). Where arthroconidia were observed to have germinated, the resulting infection hyphae were observed on several occasions to penetrate the host via stomata (Fig. 6B). Other routes of entry into host tissues were not observed.

SEM did not reveal the presence of propagules of any other fungi on the inflorescences but nevertheless, attempts were made to assess the relative virulence of the isolates of *M. scaettae*. Infection of date palm spathes under controlled conditions is very difficult, therefore we develop an infection system involving a heterologous host, the model plant *Arabidopsis thaliana*. Arthroconidia (5 µl droplets of 1 x 10^5.ml^{-1} suspension in PDB) were applied to the adaxial surface of attached leaves of Arabidopsis Col-0.

Within 6 days (144 h), necrotic lesions formed on the adaxial side of the leaf and these had penetrated through to the abaxial lamina. Such symptoms were reminiscent of the nectrophic infection phenotypes seen with *Botrytis cinerea* (Mur *et al.,* 2012). In line with this, a series of lesion phenotype scores were derived based on the extent of necrosis and chlorosis and the relative penetration through to the abaxial side of the leaf (Fig. 5C and 5D). To assess the relative virulence of each strain, arthroconidial suspensions at different concentrations (, 1 x 10^4.ml^{-1} and 1 x 10^5.ml^{-1}) were used to inoculate *Arabidopsis* leaves as described above. These were scored after 24, 28, 72 and 144 h and the mean values used to in a hierarchical cluster analysis and the derivation of a heat map (Fig. 6E). This indicated that different strains did differ in their aggressiveness on *Arabidopsis*. However, the patterns of strain aggressiveness did not correlate with origins from male or female flowers or FTIR clustering.

The development of the heterologous system allowed the further analysis of the *M. scaettae* infection system. Aniline Trypan? blue staining of infected areas suggested that arthroconidial germinate to form infection hyphae which; as with homologous hosts, penetrated the host via stomata (Fig. 5F). Once within the plant, aniline blue epifluorescence showed? the formation of a swollen appressorial-like structure in the sub-stomatal cavity (Fig. 6G).

DISCUSSION

Reports of the occurrence of other fungi associated with these symptoms *Fusarium* sp, Alternaria sp *Trichothecium sp*, and *Thielaviopsis* sp (Brown and Butler, 1938; El-Behadili et al.,1977; Rattan and Al-DBoon, 1980;Khairy et al., 1984; Hameed,2012). may relate to secondary infections or possibly a complex of aetiological agents. However, in our investigations only mycelia identical to *M. scaettae* were observed or isolated. *M. scaettae* was isolated from both male and female inflorescences. The results obtained by observing the symptoms, characteristics of pathogen fungus on Petri dishes containing the PDA and microscopic characterization has presented the inflorescence rot disease in date palm in Algerian South East oases.

Through the symptoms of the disease on all parts of the inflorescences, which includes brownish or rusty areas develop on the non-opened spathe after the pathogen has already invaded the floral tissues. Lesions may be confluent and are mostly common near the top of the spathe, which, is soft and still hidden in the leaf base at the time of infection. The internal face of the spathe under the lesions is yellow and translucent and may have brown dots corresponding to points of contact with diseased flowers. The pathogen attacks flowers and strands and may move on to the stalk of the inflorescence. Spathes, severely damaged when young, may remain closed; however, the spathe usually splits and reveals partial to near complete involvement of the flowers and strands. In the advanced infection, white mycelium of *M. scaettae* was observed around the flowers in a clear manner. The symptoms were shown agree with previous reports (Cavara, 1925 a, b; Chabrolin, 1928; Al-Ani et al., 1971; Al-Roubaie et al., 1987; Abdullah et al., 2005).

Inflorescence rot is most in severe areas with excessive or prolonged winters and spring rains, on neglected palms and in marginal areas of oases, or in waterlogged soil, salty depressions,. Male palms frequently are grown in these marginal areas as communal property pollen sources. They may be heavily infected because they receive little attention and the old inflorescences are not removed regularly. Pollination has been implicated in the movement of *M. scaettae* from diseased to healthy trees. Inflorescence rot does not spread rapidly. It is presumed that *M. scaettae* survives in old tissues as mycelium. Since spores are short lived, they are not considered to be important for the persistence of the pathogen. This is identical to what the authors pointed out (Chabrolin, 1928; Maire and Werner, 1937; Munier,1952; Munier, 1955; Calcat, 1959; Rattan and AL-Dboon 1980).

Typically, some palms develop symptoms every year, whereas others in the same planting develop symptoms only occasionally, even under favourable conditions.

Colonies of *M. scaettae* were observed on the PDA with poor white growth, up to 04 cm (Diameter) after 10 days of growth at 22 ^{0}C. White Mycelium immersed and superficially composed of branched hyaline septate hyphae. Reverse, at first creamy to pale brown becoming black in old cultures (Cavara, 1925a ; van der Walt and Hopsu-Havu, 1976; von Arx et al., 1982).

The light microscopic micrograph of the mycelium and artroconidia have shown that *M. scaettae* represents an anamorph of an unknown ascomycetes (van der Walt and Hopsu-Havu, 1976; von Arx et al., 1982).

Such attributions were made before the advent of modern molecular approaches to fungal identification. Therefore, given the supposed mixture of fungi that have been identified on infected inflorescences, *M. scaettae* needs to be both identified and linked to disease. Completion of these tasks would facilitate the development of appropriate monitoring programmes, targeted fungicide use and the long term replacement of date palm stands with resistant germplasm.

Many attempts have been made to design high-coverage primers for amplification of the fungal ITS region (White et al., 1990 ; Martin and Rygiewicz, 2005). To date, more than 90,000 fungal internal ITS region sequences (Seifert, 2009; Schoch et al., 2012), have been deposited in public databases (Hibbett et al., 2011). Several ‘‘classic’’ primers for example, ITS1 and ITS4 were developed by (White et al., 1990; Gardes and Bruns,1930) are commonly used for ITS-based barcoding of a variety of fungal groups (Manter and Vivanco, 2007), promoting various taxonomic (Sato, 2007) and ecological (O’Brien et al., 2005; Wallander et al., 2010) studies. Moreover, these can be employed in next-generation sequencing based on high-throughput DNA barcoding, as demonstrated by metagenomic studies of fungal communities in various environmental samples, such as mycorrhizae, leaves, and soil (Jumpponen and Jones, 2009; Lumini et al., 2009; Lekberg et al., 2011).

In this study, due to logistical restraints, we employed a strategy where fungal strains were isolated from diseased date palm inflorescences. The isolates thus obtained remarkedly consistent in appearance irrespective of sampling location or flower sex. Light microscopic analyses suggested mycelial development that was consistent with each strain being *M. scaettae* and SEM of the spathe showed that they were covered with arthroconidia which were similar to those expected of *M. scaettae.* Unequivocal identification of each fungal strain was provided by ITS barcoding where each exhibited very high identify to the only *M. scaettae* ITS barcode in GenBank.

This suggested negligible sequence variation between all Algerian strains except OU2 and the canonical Spanish *M. scaettae* strains. The *M. scaettae* clade formed a distinct group within a complex of *Phaeosphaeria* species. The genus *Phaeosphaeria* is large and heterogeneous that crucially include many species that are associated with monocot plants; some as pathogens. However, the relationship of between *Septoria*, *Stagonospora* and Phaeosphaeria has now been extensively defined (Quaedvlieg *et al.,* 2013); that is clearly distinct from *Leptosphaeria (*Camara *et al.,* 2002; *Figure 3).* Quaedvlieg *et al.,* (2013) showed that *Septoria* (causal agent of *S. nodorum* blotch of cereals; now named *Parastagonospora)* is a distinct genus in the family Phaeosphaeriaceae. Further genetic and morphological characterisation is required to place *M. scaettae* as a distinctive taxon within the Phaeosphaeriaceae, where our studies have suggested it could be placed.

Considering variation within *M. scaettae,* we sought to employ an approach which highlighted chemical and therefore possibility functional differences between the strains. FTIR chemical fingerprinting has been extensively used to identify bacterial species (Ojeda *et al.,* 2012) including clinically relevant pathogenic forms (Dziuba et al., 2007; Zarnowiec et al*.,* 2015). Similar efforts are showing the utility of FTIR in fungal classification (Santos et al., 2010; Lecellier et al., 2014 a ; Lecellier et al., 2014b).

Our use of FTIR further suggested the utility of this approach in fungal classification as it demonstrated; as with the ITS barcoding that strain OU2 was the most distinctive (Figure 4B). However, further discrimination was observed amongst the other strains where metabolite fingerprints appeared to show difference between strains originating from male and female flowers. This could reflect some specialisation required to infect particular types of spathe, possibly through the production of certain toxins. How far this could reflect genomic differences is currently unsure but *M. scaettae* does not appear to be particularly subtle flower infecting pathogen. According to the classification of flower infecting fungal species by Ngugi and Scherm (2006); *M. scaettae* conforms to group 1 i.e. unspecialized pathogens causing necrotic symptoms such as blossom blights. Alternatively, this separation could reflect a metabolic memory of infecting different flower organs. If this were the case, this memory was maintained over successive rounds of culture on PDA; possibly through some epigenetic imprinting.

Beyond establishing the predominance of *M. scaettae* on infected date palm inflorescences, we sought to adopt conform to the requirements of Koch's postulates and demonstrate the virulence of our isolated strains. Ideally, this should have involved the reinfection of date palm but this was not possible. Therefore, we attempted to exploit the model plant *Arabidopsis thaliana.* Somewhat surprising, *M. scaettae* was able to infect *Arabidopsis* leaves and penetrate through the leaf lamina from the adaxial to abaxial sides. Further, light microscopic analyses suggested that plant cell death was quite a delayed response, suggesting that we were not simply observing a non-host hypersensitive response to *M. scaettae*. Indeed, such observations were consistent with *M. scaettae* being a rather non-specialised fungal pathogen as suggested above. Such a suggestion has consequences for *M. scaettae* management as it seems likely that date palm is not its only host and other species could be disease reservoirs for date palm rots.

ii) *Mauginiella scaettae* is a hemibiotroph which penetrates the host via stomata

Considering the mechanism through which *M. scaettae* infects the host, this is consistent a hemibiotrophic strategy. In both homologous and heterologous hosts, *M. scaettae* seems to target stomata in order to penetrate its host. Such a strategy is also a feature of biotrophic Basidiomycetes pathogens such as rusts, were after spores land on the leaf surfaces, a germ tube forms which searches for the stomata. This then forms an appressorium over the stomatal guard cells to penetrate the stomata and form a microscopically visible haustorium in the substomatal cavity (Chong *et al.,* 1986). Similarly, with *M. scaettae* there appears to be the formation of pronounced haustorium which appears to be functional in the short term prior to the initiation of necrotrophic phase which clearly is predominant in both homologous and heterologous hosts.

Taking our observations together, *M. scaettae* is shown to be a virulent broad host range hemibiotrophic pathogen. It is related to the Phaeosphaeriaceae which includes many monocot-associated fungi. Thus, management of inflorescence rot should focus not only on date palm but in good phytosanitary practices in oases that should also include associated plant species.

REFERENCES

Abdullah S. K; Asensio L.; Monfort E.; Gomez-Vidal S.; Palma-Guerrero J.; SalinasJ.;Lopez-Llorca L. V.; Jansson H.-B. and Guarro J. (2005). Occurrence in Elx, SE Spain of inflorescence rot disease of date palms caused by *Mauginiella scaettae.* J. Phytopathol. 153:417-422.

Abu-Yaman I.K.and Abu- Blam H.A. (1971). Major diseases of cultivated crops in the central province of Saudi Arabia: I. Disease of fruit trees. Z. Pflanzenkrankh. 78:607-611.

Al-Ani H.Y.; El-Behadili A.; Majeed H.A. and Majeed M. (1971). Reaction of date palm cultivars to inflorescence rot and persistency and spreading of the disease. Phytopathol. Mediterr. 10:57-62.

Al-Hassan K.K. and Waleed B.K. (1977). Biological study on *Mauginiella scaettae* Cav. The cause of inflorescence rot of date palms in Iraq. Yearbook Plant Prot Res Min Agric. Agrar. Ref. Iraq 1:223-236. (in Arabic).

Al-Roubaie JJ, Hama N.N., Al-Hassan KK. (1987). Studies on spread of inflorescence rot and susceptibility of some male palm cultivars to the disease. J Agric. Water Resour Res. 6:67-79 (in Arabic).

Allwood J.W., Ellis D.I., Heald J.K., Goodacre R, Mur L.A.J. (2006). Metabolomic approaches reveal that phosphatidic and phosphatidyl glycerol phospholipids are major discriminatory non-polar metabolites in responses by *Brachypodium distachyon* to challenge by *Magnaporthe grisea*. Plant J. 46: 351-368 von Arx J. A. von; Walt J. P. and Liebenberg N.V.D. W. (1981). On *Mauginiella scaettae*. Sydowia, 34:42-45.

Benziouche S.E. (2012). Analyse de la filière de dattes en Algérie, constats et perspectives de développement. Etude du cas de la Daïra de Tolga. Thèse Doc en agronomie, ENSA El- Harrach.Algerie.

Brown J.G. and Butler K.D. (1938). Inflorescence blight of the date palm. J. Agric. Res. 57:313-318.

Boyes D.C. ; Zayed A.M.; Ascenzi R; McCaskill A.J. ; Hoffman, N.E.; Davis K.R. and Görlach J. (2001). Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants, The Plant Cell. 13(7): 1499-1510.

Bouguedoura N.; Ibrahim A.; Ould Mohamed A.; Saker M. and Trifi M. (2008). A paper presented at NEPAD Biotechnology Workshop "Challenges for North Africa and Promises for a Regional Integrated Program", Ezzahra, Tunisia, in collaboration with the Tunisian Society of Microbiology, PP:22-25.

Calcat A. (1959) Diseases and pests of date palm in Sahara and North Africa. FAO. Plant Prot. Bull. 8:5- 10.

Camara M.P.; Palm M.E.; van Berkum P. and O'Neill N.R.(2002). Molecular phylogeny of *Leptosphaeria* and *Phaeosphaeria*. Mycologia. 94(4):630-40.

Chabrolin C. (1928). La pourriture de inflorescence du palmier dattier. Ann. Epiphyt. 14:377- 414.

Chong J.; Harder D.E. and Rohringer R. (1986). Cytochemical studies on *Puccinia graminis* f. sp. *tritici* in a compatible wheat host. II. Haustorium mother cell walls at the host cell penetration site, haustorial walls, and the extrahaustorial matrix. Canadian Journal of Botany. 64(11): 2561- 2575.

Carpenter J. B. and Elmer H.S. (1978). Pests and Diseases of the Date Palm. U.S. Department of Agriculture, Agriculture Handbook No. 527,42 p.

Cavara F. (1925a). Atrofia fiorale in *Phoenix dactylifera* L. di Cirenacia. Atti Reale Accad Naz Lincei Ser 6:65-67.

Cavara F. (1925b). *Mauginiella scaettae* Cav. nuovo ifomicete parassita della palma da datteri in virenacia. Bol. Orto.Bot. Napoli 8: 207-211.

CBOL Plant Working Group (2009). A DNA barcode for land plants. Proc. Natl. Acad. Sci. USA. 106: 12794-12797.

Djerbi M. (1982). New records of date palm disease in the United Arab Emirates and Bahrain. Date Palm 1:307-308.

Drummond A.J.; Ashton B.; Buxton S.; Cheung M.; Cooper A.; Duran C.; Field M. ; Heled J. Kearse M. ; Markowitz S. Moir R. ;Stones-Havas S.;Sturrock S. ; Thierer T. ; Wilson A.(2012). Geneious v5.5.6, http://www.geneious.com/

Dziuba B.; Babuchowski A.; Nalecz D. and Niklewiczb M. (2007).Identification of lactic acid bacteria using FTIR spectroscopy and cluster analysis. International Dairy Journal 17: 183-189.

El-Baker A. J.(1962). Iraqi dates and varieties. Publication of the Iraqi Ministry of Agriculture, Government Press, Baghdad, Iraq. (In Arabic).

-El-Behadili A.H.; Mawlood K.A. and Diwan M.M. (1977). A New Pathogen-causing Inflorescence Rot of Date Palm in Iraq. 4th Iraqi Biol. Soc. Conf. Baghdad, 20-22. September (Abstract).

FAOSTAT, 2009. Crop Production 2008, Statistics Division, Food and Agriculture Organization of the United Nations.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39,783-791.

Gardes M.; Bruns T.D. (1993). ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

Gidman E.; Goodacre R.; Emmett B.; Smith A.R. and Gwynn-Jones D.(2003). Investigating plant-plant interference by metabolic fingerprinting, Phytochemistry 63: 705-710.

Goodacre R.; Broadhurst D. ; Smilde A.K. ; Bruce S. Kristal B. S.; Baker J. D. ; Beger R. ; Bessant C.; Connor S.; Capuani G. ; Craig A. ; Ebbels T.; Kell D. B.; Manetti C. ; Newton J. ; Paternostro G.; Somorjai R.; Sjöström M.; Trygg J. and Wulfert F.(2007). Proposed minimum reporting standards for data analysis in metabolomics. Metabolomics. 3:231-241

Guindon S.; Dufayard J.F.; Lefort V.; Anisimova M., Hordijk W.; Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59(3):30721. doi: 10.1093/sysbio/syq010. Epub 2010 Mar 29.

Hameed M. A.(2012). Inflorescence rot disease of date palm caused by *Fusarium proliferatum* in Southern Iraq. African Journal of Biotechnology. 11(35): 8616-8621.

Hibbett D.S.; Ohman A.; Glotzer D.; Nuhn M. and Kirk P. (2011). Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biol. Rev. 25: 38-47.

Hussain F. (1958). Occurrence of date palm inflorescence rot in Iraq. Plant. Dis. Rept.42:555.

Katoh K.; Misawa K. ; Kuma K. and Miyata T. (2002). A novel method for rapid multiple sequence alignment based on fast Fourier transform Nucleic Acids. 30(14):3059-3066.

Khairy S.M.; El-Meleigi M.A. and Ibrahim G.H.(1984). Inflorescence rot (Khamedj) of date palm, caused by *Mauginiella scaettae* and *Alternaria* sp. in Gassim area. Proc. Saudi Biol.
Soc. 7: 19-29.

Kiss L. (2012). Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for Fungi. PNAS, 109 (27).

Johnson C.; Boden E. and Arias j. (2003). Salicylic Acid and NPR1 Induce the Recruitment of *trans*-activating TGA Factors to a Defense Gene Promoter in Arabidopsis, The Plant Cell. 15(8): 1846-1858.

Jumpponen A. and Jones K.L. (2009). Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytol. 184: 438-448.

Lekberg Y.; Schnoor T.; Kjoller R.; Gibbons S.M. and Hansen L.H (2011). 454 sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. J. Ecol. 100: 151-160.

Lecellier A. ; Mounier J. ; Gaydou V.; Castrec L.; Barbier G.; Ablain W.; Manfait M. ; Toubas D. and Sockalingum G.D. (2014a). Differentiation and identification of filamentous fungi by high- throughput FTIR spectroscopic analysis of mycelia. International Journal of Food Microbiology 168-169 : 32-41.

Lecellier A. ; Gaydou V.; Mounier J. ; Hermet A.; Castrec L.; Barbier G.; Ablain W.; Manfait M. ; Toubas D. and Sockalingum G.D.(2014b).Implementation of an FTIR spectral library of 486 filamentous fungi strains for rapid identification of molds. Food Microbiology 30:1-9.

Lumini E.; Orgiazzi A.; Borriello R. ; Bonfante P. and Bianciotto V. (2009). Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Env. Microbiol. 12:2165-2179.

Maire R. and Werner R. G. (1937). Fungi Maroccani. Mem. Sc. Nat. Maroc. 45: 1-147.

Manter D.K. andVivanco J.M. (2007). Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. J. Microbiol Methods 71: 7-14.

Martin K.J. and Rygiewicz P.T. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol. 5:28.

MacFie H.J.H.; Gutteridge C.S. and Norris J.R. (1978) Use of canonical variates in differentiation of bacteria by pyrolysis gas-liquid chromatography. Journal of General Microbiology 104, 67-74.

Michael I.F. and Sabet K. A. (1970). Biology and control of *Mauginiella scaettae* Cav., the pathogen of Khamedj disease in the United Arab Republic. Ann. Date Grower's Inst.47:5-8.

Munier P. (1955). Le palmier dattier en Mauritanie. Ann. Inst. Fruits et Agrumes Coloniaux 12:66.

Munier P. (1952). L'assaba, essai monographique, Saint- Louis, Senegal, Centre ifanmauritanie. Études mauritaniennes No. 3, 72 pp.

Mur L. A. J.; Sivakumaran A.;, Julien Mandon J.; Cristescu S.M., Harren F.J.M. and Kim H. Hebelstrup K.H. (2012). Haemoglobin modulates salicylate and jasmonate/ethylenemediated resistance mechanisms against pathogens.Journal of Experimental Botany, 63(12):4375-4387.

Ngugi H.K. and Scherm H. (2006). Biology of flower-infecting fungi. Annu. Rev. Phytopathol. 2006;44:261-82.

O'Brien H.E.; Parrent J.L.; Jackson J.A.; Moncalvo J.M. and Vilgalys R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. Appl. Env. Microbiol. 71: 5544- 5550.

Ojeda J.J. and Dittrich M. (2012). Fourier transform infrared spectroscopy for molecular analysis of microbial cells. Methods Mol Biol. 2012;881:187-211.

Phookamsak, R., Liu, J. K., McKenzie, E. H., Manamgoda, D. S., Ariyawansa, H., Thambugala, K. M., Dai, D-Q., Camporesi, E., Chukeatirote, E., Wijayawardene, N.N., Bahkali, A.H., Mortimer, P.H., Xu, J-C. and Hyde, K.D. (2014). Revision of Phaeosphaeriaceae. *Fungal Diversity*, *68*(1), 159-238.

Rattan S. S. and AL-Dboon A. H. A. (1980). Notes on fungi associated with date palm. I. Sydowia 32: 246-273.

Routledge A.P.M.; Shelly G.; Smith J.V.; Talbot N.J.; Draper J. and Mur L.A.J. (2004). *Magnaporthe grisea* interactions with the model grass *Brachypodium distachyon* closely resemble those with rice (*Oryza sativa*). Mol. Plant. Pathol. 5: 253-265.

Phookamsak R. ; Liu J.K. ; McKenzie E.H.C. ; Manamgoda D.S. ; Ariyawansa H. ; Thambugala K.M. ; Dai

D.Q. ; Camporesi E. ; Chukeatirote E. ; Wijayawardene N.N. ; Bahkali A.H. ; Mortimer P.E. ; Xu J.C. and Hyde K.D.(2014). Revision of Phaeosphaeriaceae. Fungal Diversity 68:159-238.

Ploetz R.C. (2003). Disease of date(Chapter 9 in Diseases of tropical fruits crops). CAB publishing, British library, London, UK Library of Congress, Washington, DC, USA. PP: 235- 236.

Quaedvlieg W.; Verkley G.J.; Shin H.D.; Barreto R.W. ; Alfenas A.C.; Swart W.J.; Groenewald J.Z. and Crous P.W. (2013). Sizing up Septoria. Stud. Mycol. 75(1):307-90.

Santos C.; Fraga M.E.; Kozakiewicz Z. and Lima N. (2010). Fourier transform infrared as a powerful technique for the identification and characterization of filamentous fungi and yeasts. Res. Microbiol. 161(2):168-75.

Seifert K.A. (2009). Progress towards DNA barcoding of fungi. Mol. Ecol. Res. 9: 83-89.

Schoch C.L.; Seifert K.A.; Huhndorf S.; Robert V. and Spouge J.L., André Levesqueb C.; Chenb W. and Fungal Barcoding Consortium (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proc. Natl. Acad.Sci. USA*.109:6241-6246.

Taylor J. W. ; D. J. ; S. KrokenS.; Takao Kasuga T. ; D. M. ; GeiserD. M. ;HibbettD. S. and FisherM. C. (2000). Phylogenetic Species Recognition and Species Concepts in Fungi. *Fungal Genetics and Biology* 31:21-32.

Teisseire M. (1961). Les ennemis du palmier-dattier et de la datte: Mesures à prendre pour les combattre. In:-Journées de la Datte, 34 Mai, 1961, Direct. Dept. Serv. Agri., Aurès, Algiers, 47-58.

Timmins E.M. ; Howell S.A.; Alsberg B.K. ; Noble W.C. and Goodacre R.(1998). Rapid differentiation of closely related *Candida* species and strains by pyrolysis-mass spectrometry and Fourier transform-infrared spectroscopy, J. Clin. Microbiol. 36(:367-374.

Toju., H.; Akifumi ; Tanabe S. ; Yamamoto S. and Sato H. (2012). High-Coverage ITS Primers for the DNA-Based Identification of Ascomycetes and Basidiomycetes in Environmental Samples. PLOS ONE, 7:01-12.

Touzi A. (2007). Algerian experience in preserving fragile ecosystems from desertification, a paper presented at the “Fifteenth OSCE Economic and Environmental Forum - Part 2: “Key challenges to ensure environmental security and sustainable development in the OSCE area: Land degradation, soil contamination and water management” Prague, 21 - 23 May 2007. Session IV: Challenges to the management of water resources and to countering desertification in the Mediterranean region, Organization for Security and Cooperation in Europe Secretariat.

Wallander H.; Johansson U.; Sterkenburg E.; Durling M.B. and Lindahl B.D. (2010). Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. New Phytol. 187: 1124-1134.

White T.J.; Bruns T.; Lee S. and Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A; Gelfand H.; Sninsky J.S.and White T.J. Eds. PCR Protocols: a guide to methods and applications. San Diego: Academic Press. pp 315-322.

Windig W.; Haverkamp J. and Kistemaker P.G. (1983) Interpretation of sets of pyrolysis mass spectra by discriminant analysis and graphical rotation. Analytical Chemistry 55: 81-88.

Zarnowiec P.; Lechowicz L.; Czerwonka G. and Kaca W. (2015). Fourier Transform Infrared Spectroscopy (FTIR) as a Tool for the Identification and Differentiation of Pathogenic Bacteria. Curr. Med. Chem. 22(14):1710-8.

Table

Table 1. Ten sites in South-East of Algeria origin of the inflorescence rot in date palm.

Sample Origin	Grid Reference	Isolate code	Date palm variety	Sex	Tree Age (years)	GenBank Accession
Biskra	N34.84, E5.72	BI	Doker	Male	52	KT587190
El Hadjira	N32.62, E5.51	HA	Doker	Male	30	KT587192
El Oued	N33.37, E6.84	OE1	Ghars	Female	60	KT587191
El Oued	N33.37, E6.84	OE2	Ghars	Female	65	KT587187
Ghardaia	N32.51, E3.63	GA	Ghars	Female	35	KT587194
Ouargla	N31.96, E5.31	OU1	Doker	Male	50	KT587185
Ouargla	N31.96, E5.31	OU2	Doker	Male	36	KT587186
Ouargla	N31.96, E5.31	OU3	Doker	Male	45	KT587193
Touggourt	N33.10, E6.00	TO1	Doker	Male	40	KT587188
Touggourt	N33.10, E6.00	TO2	Doker	Male	47	KT587189

Figures

Supplementary Fig. 1. Sampling sites in South-East of Algeria origin of the inflorescence rot in date palms ((blue dots) and site of only previously genetically characterised strain (red dots).

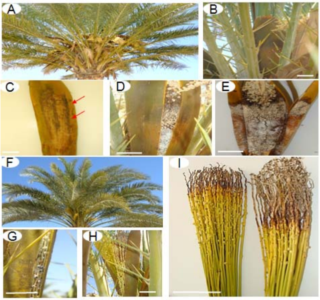

Fig. 1. Symptoms of date palm inflorescence rot caused by *Mauginiella scaettae*. **(A)** Male date palm with **(B)** male inflorescences with the spathe cover (bar=10 cm)**. (C)** Necrotic symptoms symptomatic of inflorescence rot as seen on the outside of the male spathe (bar=5 cm) with **(D)** white mycelial development within the male spathe (bar=2 cm). **(E)** white mycelial development and necrosis is eventually observed both within and outside the infected male spathe (bar=5 cm). **(F)** healthy female date palm exhibiting the emergence of female flowers. **(G)** white mycelial development within inflorescence rot exhibiting female spathe (bar=2 cm) which **(H)** is associated with necrotic symptoms as the flowers emerge (bar=10 cm) and **(I)** kills the flowers at the tips (bar=5 cm).

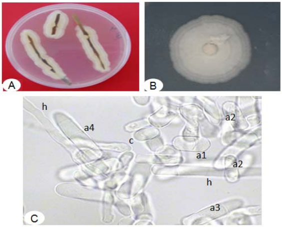

Fig. 2. Culture and light microscopic analysis of fungal strains isolated from diseases male and female date palm spathe. **(A)** The emergence of white fungal mycelia from explants of inflorescence rot exhibiting spathe. **(B)** After many rounds of sub-culture, strains of white fungal colonies were isolated. **(C)** light microscopic analysis of fungal strain morphologies showing hyphae (h), and one (a1), two (a2), three (a3) and four-celled (a4) arthroconidia.

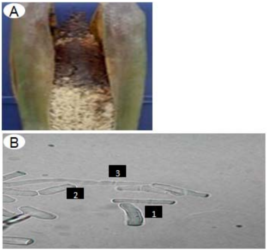

Fig. 3. Date palm spathe 1 weeks after artificial inoculation with *Mauginiella scaettae* (strain GA). **(A)** Opened spathe showing symptoms at the top. **(B)** Pure culture *Mauginiella scaettae* was re-isolated from all artificially inoculated parts: 1- from stand, 2-from flower, 3- from spathe. **(C)**Light microscopic analysis of GA strain morphologies showing hyphae and arthroconidia.1- Arthroconidia tow cells, 2- Arthroconidia three cells, 3- Hyphae (100x).

Fig. 4. Maximum likelihood tree based on ITS sequences (546 bp alignment) for *Mauginiella* and other members of the family Phaeosphaeriaceae using *Alternaria* and *Stemphylium* (Pleosporaceae) as outgroup taxa. The backbone of the tree and clade assignation are based on the data of Phookamsak et al. (2014). Values at nodes indicate bootstrap percentages (1000 replicates) which exceed 50%.

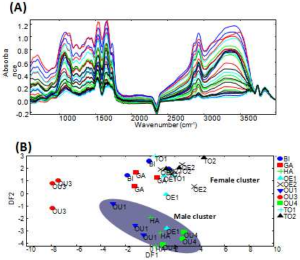

Fig. 5: Metabolite fingerprinting *Mauginiella scaettae* isolates using FTIR spectroscopy. **(A)** Typical FTIR spectra obtained from mycelia of *M. scaettae* strains **(B)** Discriminant Function Analysis (DFA) of FTIR spectra based on 4 principal components (PC) explaining 96.5% of the total variation. Two clusters are indicted encompassing fingerprints obtained from either female (green) or male (orange) spathe.

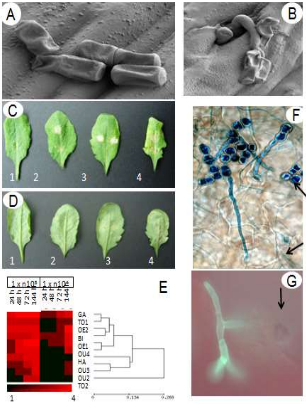

Figure 6: The infection strategy of *Mauginiella scaettae* on homologous and heterologous hosts. Scanning electron microscopy (SEM) micrograph of the surface of inflorescence rot exhibited date palm showing **(A)** distinctive arthrocondia and **(B)** a germinated arthrocondium demonstrating penetration of the host via a stoma. Development of *Arabidopsis thaliana* as a heterologous host for *Mauginiella scaettae.* Infection scores for adaxial spot inoculation of *Arabidopsis* showing phenotypes on the **(C)** adaxial and **(D)** penetration through to the abaxial lamina. **(E)** Mean infection scores for spot inoculations (n=10) for *M. scaettae* strains (**Table 1**) displayed using a heat map and hierarchical cluster analyses. **(F)** aniline blue staining of germinating *M. scaettae* arthroconidia demonstrating targeting of stomata (arrowed). **(G)** epifluorescent analine blue staining showing penetration of an *Arabidopsis* stoma by *M. scaettae* (arrowed) with the development of a sub-stomatal haustorium.

Promoting the Application of ICT Tools in Management Programs of Date Palm Pests in Arab countries

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Abstract

ICT is one of the emerging technologies worldwide, successfully used in many agricultural purposes in general. The objective of this paper is to promote the utilization of this technology in the Arab countries for the sustainable development of date palm plantations, particularly with regard to pest management. The paper will provide a brief introduction to available ICT tools and discuss their applicability to support pest management programs in the near future. This discussion will focus on the features, characteristics and forms of services, use and application mechanisms, operational directions for development, challenges/constraints of application, and specific sites of the key players of ICT. Finally, special attention will be given to the application of these technologies in order to demonstrate their potential impacts on: (1) Providing information on the infestation by various pests from specialized sites and databases, (2) Determination of the location and extent of infection in certain areas using GPS&GIS, and early detection of some serious pests such as red palm weevils, (3) Exchange of information on the best measurements and control tactics to the pest of concern, (4)Supporting pest management decisions and enhancement of the evaluation of the adopted IPM programs, (5) Establishing expert systems for management of date palm pests, (6) Strengthen the communication links between specialized researchers, centers, extension professionals, date palm growers, traders and agro-industrialists, and others (7) Rapidly changing and technology transfer of research and extension information to producers and date palm growers.

Keywords: Date palm, pests, IPM, ICT tools, management decisions.

INTRODUCTION

The integration of information processing and telecommunications is identified at the present time as Information communication technology (ICT). Such technology refers to systems for producing, storing, sending and retrieving different contents of digital files (text, sounds and images). It is well known that digital files with suitable equipment can be stored indefinitely without any loss of quality, they can be reproduced at almost no cost, and they can be transmitted almost instantaneously over great distances (Bartlett, 2002).The most commonly ICT takes the physical form of a desk-top computer connected to the internet by means of a modem and a telephone line. Also, devices such as compact disk drives, digital cameras, scanners and color printers can be connected to the computer to provide additional means for inputting and outputting of data (Celebic and Rendulic, 2011).

Recently ICT tools have been used around the world to enhance sustainable agricultural development in general (Sylvester, 2013, Berti and Catherine Mulligan, 2015) and integrated pest management in particular (Bartlett, 2002; MacRae, 2002 and Azfar *et al*, 2015). Generally, the success of such tools to improve agricultural productivity and reduction of pest problems still faced in the Arab countries as well as in many foreign countries by various constraints and challenges. The actual reality showed that the widespread of ICT applications are still absent or on a very limited scale. Regarding the date palm pests only various tools of ICT in management steps were tried and or reported for some purposes. For examples by: using specialized sites and databases to provide information on the infestation of date palms by various pests in the eastern region, Al Ain, UAE (Khalifa, *et al*, 2010; SCRE, 2015), using geographic information system for assessing the activity of the red palm weevil *Rhynchophorus Ferrugineus* (Olivier) in the date palm oasis of Al-Hassa, Saudi Arabia (Massoud *et al*, 2011), developing and using expert systems for management of date palm pests in Arab region (El-Zemaity, 2007) and using an object- oriented expert system for diagnosis of fungal diseases of date palm (Al-Ahmar, 2009).

With taking into consideration the great importance that the Arab countries concerned to dates palm, the objective of this paper is to discuss various aspects related to the promotion and dissemination of the uses of ICT in sustainable development and management of date pests in the Arab countries.

DISCUSSION

The following discussion will focus on the different aspects of ICT related sustainable development and management of date palm pests and will includes: ICT tools, characteristics and forms of services, use and application mechanisms of ICT, and specific sites of the key players. Detailed consideration will be given to the potentials of ICT tools and precision IPM practices especially global positioning system (GPS), expert systems, wireless sensor network (WSN) and the internet of things (IoT) in order to demonstrate their potential impacts on a decision support system for management of the date palm pests. Finally, it will highlight on challenges/constraints of ICT application in the Arab region and other developing countries.

ICT tools, characteristics and forms of services

ICT which could be use in sustainable development and management of date pests includes different tools such as: Telephone /fax, Computers (Personal computer -Laptop or tablet – Portable digital devices/PDA- Personal Digital Assistant, PALM), Broadcasting (digital TV - digital radio), Satellite, Mobiles (Mobile phone – Smartphone), Internet and broadband, Sensor networks and GPS (global positioning systems). Some of these tools are inexpensive and affordable and are easily used by many individuals regardless of the level of education or culture. Therefore, with a little training it is expected to be successfully used with a wide range of farmers and date palm growers as well as extension and plant protection specialists.

The role of the internet in developing and adoption of IPM through rapid access to IPM information were emphasized by various organizations which conjugated and released Database of IPM Resources (DIR) at year 2000 (http://www.ipmnet.org/cicp/). Internet characteristics which make it highly suitable as an important source of information were summarized by Bartlett, 2002 as follows:

1- The contents of a website can be revised far quickly, and the delivery of the new content is immediate.

2- The internet can be accessed at any time.
3- The vast scope of the internet makes it possible to find details which are relevant to the specific needs.
4- There are very few controls on who can use the internet, both as senders or receivers. The internet doesn't stop people from accessing information because of their sex, race, class or age (can be created by governmental authorities, commercial and NG organizations, associations, community groups and individuals).
5- On-line discussion groups can be used to share experience, and some websites offer an 'ask an expert' service; the number of active participants in these forums can be far greater than – say - a radio programs.

Available products of ICT take different types of services such as: Email messages, discussion groups, On-line databases and libraries**,** Web-based resource centers (news, training materials, directories of organizations and),Organizational home pages (Government Departments, NGOs and development projects), Training and reference materials on compact disk (text, video and sounds can be attractively integrated) and Distance-learning courses (These courses can combine the benefits of CDs, websites, and Email). Due to the importance of these services to different sectors of farmers, workers and specialists in the field of dates, there is no doubt that there is a need to play government agencies and non-governmental organizations interested in the production and marketing of dates an active role in the dissemination of ICT and use, especially in the areas of education, extension and training.

Use and application mechanisms of ICT

Mechanisms of ICT that could be used to improve the flow of information in general were identified by Sithole *et al*, 1998. Based on this valuable identification, ICT mechanisms that could be used in the field of sustainable development and management of date palm pests can take different activities such as: chatting for brain storming and group meetings over the computer, broadcasting e-mails to experts for experience exchange, discussions group development that would provide access to both national and international interaction to all members of the discussions groups, data and paper publishing without costly printing charges, web-mining for information on related (and unrelated) sites would provide much of the basic graphic and biological information sought and web-page publishing by local projects so that field programs and research institutions can begin to develop meaningful partnerships. Use of different ICT tools in IPM in general is illustrated in Fig.1, and the application of some of these tools in management pests of date palm in particular is showed in Table (1).

Specific sites of the key players of ICT related sustainable development and management of date palm pests

1- AARINENA Regional *Date-palm* Network
www.aarinena.org/aarinena/networks/dp/index.asp
2- Early Detection of Red Palm Weevil Infestations Using Drones and Advanced Aerial Data Analysis Tools
www.dronesforgood.ae/.../early-detection-red-palm-weevil-infestatio...
3- Date - Date palm - World Crops Database - Tropical fruits
world-crops.com › Crops
4- Genome sequence of the date palm *Phoenix dactylifera* L

https://www.nature.com/articles/ncomms3274
5- Date palm - USDA Plants Database
plants.usda.gov/java/profile?symbol=phda4
6- Date Palm Research Center of Excellence (DPRCE), King Faisal University
https://www.kfu.edu.sa/ar/Centers/palms/Documents/DPPP_Research_Program%20revised.pdf
7- Development of Sustainable Date Palm Production Systems in Gulf Cooperation Council Countries,
International Center for Agricultural Research in the Dry Areas (ICARDA)
file:///C:/Users/Said/Downloads/Development_of_date_palm.pdf
8- Pinterest Date palms
https://www.pinterest.com/explore/date-palms/
9- Facts and Details - Dates and Date Palm Cultivation
http://factsanddetails.com/world/cat54/sub343/item1574.html
10- RAMBOLL - Arabian Date Palm Tree Pole
http://www.ramboll.com/projects/rt/arabian%20date%20palm%20tree%20pole
11- Iraqi Date Palms
http://www.iraqi-datepalms.net/Web/WebSubContent.aspx?id=164
12- 100 Dates – Purugganan Laboratory,
Puruggananlab.bio.nyu.edu/index.php?action=100Dates
13- Red Palm Weevil Website
http://www.redpalmweevil.com/

Potential of ICT tools and precision IPM practices

1.Global Positioning System (GPS) and Geographic Information System (GIS) - The wide spread of the applications of Geographic Information System (GIS) and Global Positioning System (GPS) in different objects of our life and the important role of using such systems in precision agriculture in general and in pest management in particular were highly reported. Actually, GPS receivers become cheap and more popular for many uses and provide benefits in map-making with high accuracy and surveying of pests in interested locations. Whereas, GIS can serve as a valuable tool in area-wide IPM programmes and it is ideally suited for managing data on the nature, location and spread of pests. On the other hand, the system provides vast capabilities for tracking, and predictive analysis of many pests. Regarding of RPW, Massoud *et al*, 2011 stated that GIS based techniques were used to study the spatial spread of RPW in the date palm oasis of Al-Hassa, Saudi Arabia. Also, GIS has been used to keep track of RPW infested palms in several countries, including Egypt and Spain (Barranco *et al*. 2006 and Brun *et al*. 2006).

2. **Expert systems -** Expert Systems in the field of pest management can employs human knowledge captured in a computer to solve problems that ordinarily require human expertise of different disciplines. Expert systems can facilitate knowledge transfer and can guide growers to take decision into different aspects of crop management for increasing the productivity and the profit margin and also combines the experimental and experiential knowledge with the intuitive reasoning skills of a multitude of specialists to aid farmers in making the best decisions for their crops (Abdel-Hadi *et al*, 2006 and Dath and Balakrishnan, 2013). It is thought that the development of an expert system on date palm will help growers in faster dissemination of expert advice for different locations at the same time and will guide

them to make decision into different aspects of pest management especially pest diagnosis and implementation the most proper practices of management the target pest (El-Zemaity, 2007, MOEW, 2008, Al-Ahmar, 2009 and Kolhe & Gupta, 2014).

3. Wireless sensor network (WSN) - WSN is a designed system that checks pest infestation at early stage and notify the farmer of the crop where the infestation is taking place. Of course, this reduces the burden of farmers by preventing them to manual monitoring of the field. Pest control Mechanisms including analysis of symptoms, identification and management of pests by using WSN were reported by many researchers. For example Azfar *et al*, 2015 reviewed pest detection and control techniques using wireless sensor network and stated that the possibility using this technology in developing countries with affordable cost. However, the actual reality indicates that the use of this tool is still generally limited, and it is expected that its applications will increase in the future. The potential use of various remote sensing techniques such as spectrophotometry, spectral line imaging, spectrometry, imaging of fluorescence, thermos-radiometry and thermography as indirect methods to identify the pest's infection/ infestation was recently introduced as a promising approach. The usage of thermal remote sensing technology (thermography) through thermal imaging is gaining popularity in pest detection due to the reductions in the cost of the equipment and simple operating procedure (Al-doski *et al*, 2016). Detection methods of date palm pests especially RPW infestation including remote sensors were discussed by Al-Manie and Alkanhal, 2005, Pinhas *et al*, 2008, Gutiérrez *et al*, 2010, Rach *et al*, 2013, Srinivas *et al*, 2013, Soroker *et al*, 2013, Yones *et al*, 2014, and Mulley, 2017. However, some opinions such as Soroker *et al*, 2013 believe that remote detection of RPW by thermal and olfactory cues is very promising for area wide inspection but still far from application.

4. The Internet of Things (IoT) - In recent time a new approach of interacting physical objects embedded with electronic, software, radio-frequency identification, sensors, actuators and smart objects with the internet to accumulate and share data through an emerging technology known as the Internet of Things (IoT). The sensors monitoring the pest behavior, pest's population, and analyzes environmental parameters and plant growth. Based on this data the pesticides are automatically sprayed into the field at the place where it requires and in the right amount so that pests are disrupted initially (Shewaramani, 2015). Also, Mehta, 2015 emphasized that the Agriculture Internet of Things has a system that monitor and scans the environmental parameters and plant growth, further this data is utilized by pest control sensors which is capable of predicting pest behavior. This information can be used by farmers to reduce damage done by pests on a large scale. Again, the concept of automated remote insect surveillance at large spatial scales and the Internet of Things was discussed by Nathan, 2015, Potamitis, 2017 and control of mites by Abd EL-Wahab Rania, 2017. However, only few of published papers reported the application of such technology in monitoring plant disease and insect pests such as Shi *et al*, 2015. On the other hand, till now no available publications on using (IoT) in management of date palm pests.

Challenges/constraints of ICT tools application

There's no debate that the level of end users (farmer's/growers) education/ literacy levels plays an influential role in the extent of use available ICT tools. So, without an education (at least reading skills) or training it is difficult for farmers to send and receive information. In some countries the computer literacy could be the main limiting factor on the use of ICT in providing information on IPM to farmers especially in rural regions. On the other hand, the lack of clear ICT policies of governments, research and farmer organizations often reflect on ICT adoption. Adding to the mentioned previous, constraints of application ICT tools in Arab region and other developing countries or limit its applicability can be summarized as follows:

- Cost and the required use of relatively some expensive systems.
- Difficulty of adoption and implementation of some devices especially that need power supplies.

CONCLUSION

ICT tools had a real potential that enable farmers to better monitor pest populations of date palms. In the event pest populations are reaching tolerance levels, farmers can take decisions of pest control or remotely release a control agent. Literacy levels, government funding, and computer skills are the main factors encumbering developing countries' agriculturalists from successful application of ICT tools. Efforts of all interested sectors of date palm to enhance the use of ICT tools and its applications in online recording of pest data directly in the field via mobile internet devices are required.

LITERATURE CITED

Abd El-Wahab, R.A. 2017. Internet of Things (IoT) to Control the Two-Spotted Spider Mite, *Tetranychus urticae* Koch in Greenhouses. Available at: http://www.academia.edu/35045569/

Abdel-Hadi, Z; Assad, S.; Bayaa, B.; Dahab, M.; Edris, S.; El-Azhary, S.; El-Bahnasy, K. ; El-Sayed, A. ; El-Zemaity, M.S. ; Grando, S.; Ketata, H.; Kumari, S.; Malhotra, R.; Mosaad, M.; Pande, S. ; Rafea, A.; Ragab, A.; Rao, G.V.; Said, A.; Shata. A. and Yehyaoui, A. 2006. Rapid Generation of Plant Protection Expert Systems. Proceedings of the 4th World Congress on Computers in Agriculture, American Society of Agricultural and Biological Engineers. Florida USA, July 24-26, 2006, pp: 282-287.

Al-Ahmar, M.A. 2009. An Object- Oriented Expert System for Diagnosis of Fungal Diseases of Date Palm. International Journal of Soft Computing 4(5):201-207.

Al-doski ,J., Mansor, S. and Shafri , H. Z. 2016. Thermal Imaging For Pests Detecting—A Review. International Journal of Agriculture, Forestry and Plantation, Vol. 2 (February.)

Al-Manie, M.A. and Alkanhal, M.I. 2005. Acoustic detection of the red date palm weevil. World Acad. Sci. Eng. Technol., 2: 160-163. http://waset.org/journals/waset/v2/v2-40.pdf

Azfar, S., Nadeem, A. and Basit, A. 2015. Pest detection and control techniques using wireless sensor network: A review. Journal of Entomology and Zoology Studies 2015; 3 (2): 92-99

Barranco P., Lorente-Moreno F., Alcazar-Alba M.D., and Pena-Mendez Y.J. 2006. Control of *Rhynchophorus ferrugineus* in Spain: field tests and treatments. Proc. 1st International

Workshop on Red Palm Weevil. IVIA, Valencia, Spain, 28–29, November 2005, 180 pp.

Bartlett, A.P.2002. ICT and IPM. In Farmers, FAO and Field School: Bringing IPM to the grass roots in Asia. Food and Agriculture Organisation , Available at: http://ipm-info.org/library/communityipm.htm

Berti,G. and Mulligan, C. 2015. ICT and the Future of Food and Agriculture. Imperial Collage, London, Sustainable Society Network. Telefonaktiebolaget LM Ericsson, Stockholm, Sweden.

Celebic, G. and Rendulic D. I. 2011. Basic Concepts of Information and Communication Technology, handbook. Open Society for Idea Exchange (ODRAZI), Zagreb.

Date Palm Research Center of Excellence (SCRE). 2015. Sustinable Pest Management in Date Palm (SPMDP) Project.King Faisal University, Saudi Arabia.

Dath, A., and Balakrishnan, M. 2013. Development of an Expert System for Agricultural Commodities. The International Journal of Computer Science & Applications (TIJCSA), Volume 2, No. 07, September 2013. Available Online at http://www.journalofcomputerscience.com/

El-Zemaity, M.S. 2007. Developing and Using Expert Systems for Management of Date Palm Pests in Arab Region – What Kinds of Information May Be Needed? The Fourth Symposium on Date Palm in Saudi Arabia, King Faisal University, Al-Hassa, 5-8 May 2007: 1167-1178, available at: http://188.40.152.201/Files/newbook/N4/en/files/assets/basic-html/page524.html

Gutiérrez, A.; Ruiz, V.; Moltó, E.; Tapia, G. and Del Mar Téllez, M. 2010. Development of a bioacoustic sensor for the early detection of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier), Crop Protection 29 (2010) 671-676.

Khalifa, O., El Assal, A.H.; A l Ezaby, F.A.; Murse, M.A.; Al Nuaimi, S.M. and Al Zehli, N. S. 2010. Integrated pest management for the Control of Red Palm Weevil (*Rhynchphorus ferrugineus oliv*) in the eastern region, Al Ain, UAE. Khalifa International Date Palm Award, THE BLESSED TREE - MARCH 2010:22-36

Kolhe,S. and Gupta, G. K. 2014. Intelligent Systems for Agriculture Domain, International Journal of Computer Applications (0975 – 8887) National Conference on Emerging Trends in Computer Technology (NCETCT-2014):14-18

MacRae, I.V. 2002. IPM Resources on the World Wide Web. Available at: (http://ipmworld.umn.edu/chapters/Macrae.htm)

Massoud, A.M; Faleiro, J. R.; Abo El-Saad, M. and Sultan, E. 2011. Geographic Information System Used For Assessing The Activity Of The Red Palm Weevil *Rhynchophorus Ferrugineus* (Olivier) In The Date Palm Oasis Of Al-Hassa, Saudi Arabia. Journal of Plant Protection Research Vol. 51(3): 234-239.

Mehta, Y. 2015. Agriculture Internet of Things (IoT) Technology Applications. Available at: http://iotworm.com/agriculture-internet-of-things-iot-technology-applications/

Ministry of Environment and Water (MOEW). 2008. The Provision of Expert Systems in the Programs of Serving and Protecting the Palm Trees, MOEW Project (under development). UAE. http://www.moew.gov.ae./En/pages/default.aspx.

Mulley, M. 2017. Assessing Date Palm Health Using Remote Sensing: The Case of the Red Palm Weevil. MSc thesis, Wageningen University and Research, The Netherlands. Laboratory of Geo-Information Science and Remote Sensing, GRS – 80436. Available at: http://edepot.wur.nl/424179

Nathan, To. 2015. IoT and Agriculture: How the Internet of Things is Changing Agricultural Operations. Available at: http://www.davranetworks.com/news/

Pinhas, J.; Soroker, V.; Hetzroni, A.; Mizrach, A.; Teicher, M. and Goldberger, J. 2008. Automatic acoustic detection of the red palm weevil, Computers and Electronics in Agriculture 63 (2008) 131-139.

Potamitis, I.; Eliopoulos, P. and Rigakis, I. 2017. Automated Remote Insect Surveillance at a Global Scale and the Internet of Things. Robotics 2017, 6, 19; doi:10.3390/robotics6030019. Available at: www.mdpi.com/journal/robotics

Rach, M. M.; Gomis, H. M.; Granado, O. L.; Malumbres, M. P.; Campoy, A. M. and Martín, J. J. S. 2013. On the Design of a Bioacoustic Sensor for the Early Detection of the Red Palm Weevil. Sensors (Basel). 2013; 13(2): 1706–1729. Available at: http://www.mdpi.com/1424-8220/13/2/1706

Shewaramani, M. 2015. How Internet Of Things Is Modernizing Agriculture Landscape? Available at: https://www.credencys.com/blog/how-internet-of-things-is-modernizing-agriculture-landscape/

Shi, Y.; Wang, Z.; Wang, X. and Zhang, S. 2015. Internet of Things Application to Monitoring Plant Disease and Insect Pests. International Conference on Applied Science and Engineering Innovation (ASEI 2015), 31-3, Available at: https://www.researchgate.net/.../299931851

Sithole, S.Z.; Gallagher, K.D. and Taguich, M.1998. Information Needs on Integrated Production and Pest Management Programs. Proceeding of the Integrated Pest management Communications Workshop: Eastern/Southern Africa, ICIPE, 1998 (http://www.ag.vt.edu/ail/ipmcw/proceedings/proceed.htm)

Soroker, V.; Suma, P.; La Pergola, A.; Cohen1, Y.; Cohen, Y.; Alchanatis, V.; Golomb, O.; Goldshtein, E.; Hetzroni, A.; Galazan, L.; Kontodimas, D.; Pontikakos, C.; Zorovic, M. and Brandstetter, M. 2013. Early Detection and Monitoring of Red Palm Weevil: Approaches and Challenges. AFPP – Palm Pest Mediterranean Conference Nice – 2013.

Srinivas, S.; Harsha, K.S.; Sujatha, A. and Kumar, N. 2013. Efficient protection of palms from RPW Larvae using WSN: IJCSI 2013, 10(3):2.

Sylvester,G. 2013. Information and Communication Technologies for Sustainable Agriculture -*Indicators from Asia and the Pacific*. FAO, RAP PUBLICATION 2013/14.

Yones, M. S.; Aboelghar, M. A.; El-Shirbeny, M. A.; Khdry, G.A.; Ali, A. M. and Saleh, N.S. 2014. Hyperspectral Indices For Assessing Damage By The Red Palm Weevil *Rhynchophorus Ferrugineus* (Coleoptera: Curculionidae) In Date Palms International Journal of Geosciences and Geomatics, Vol. 2, Issue 2, 2014,ISSN:2052-5591

Brun, L. 2006. The date palm in Egypt: a traditional cash crop threatened by the Red Palm Weevil. Proc. 1st International Workshop on Red Palm Weevil. IVIA, Valencia, Spain, 28– 29, November 2005, 180 pp.

Table

Table.1. Application of ICT tools in management pests of date palm

ICT tool	Application	Source
- Geographic Information System Used (GPS) - Satellite images (GIS)	- Assessing the activity of the insect pest (Red palm weevil *Rhynchophorus ferrugineus*) - Locating infested areas and incorporating the data in management programs	Massoud *et al*., 2011 SCRE, 2015
Databases	- Providing information on pest Infestation (Red palm weevil *Rhynchophorus ferrugineus)* - Providing information on both pests palm and their natural enemies	Khalifa *et al*., 2010 SCRE, 2015
World Wide Web/ Websites	- Supporting decisions of pest and disease management - Delivering and sharing IPM related information - Identifying the pest species, its damage and life history - Monitoring, scouting and predicting populations - Selecting and applying control techniques	Yulu Xia *et al*., 2007 Stinner, 1999 Bartlett, 2002
Precision Hawk's UAV and data analysis platform, Data Mapper.	Detecting and combating diseases and pest infestation (Early Detection of Red Palm Weevil Infestations Using Drones and Advanced Aerial Data Analysis Tools)	www.dronesforgood.ae /.../early-detection-red-palm-weevil-infestation
Expert Systems	- Diagnosing Date Palm Diseases (El-Askary and Baraka, Faculty of Information Technology, Islamic University, Gaza. Palestine). - Diagnosing of fungal diseases of date palm - Implementations of IPM practices and supporting management decisions - Disease diagnosis	Al-Ahmar, 2009 El-Zemaity, 2007 Kolhe, and Gupta, 2014
Mobiles (Mobile phone – Smartphone)	- Wireless communications - Taking and sharing pictures of infestation symptoms	
Sensor networks/ Wireless sensor network	Pest disease monitoring, and different pest control mechanisms.	Azfar *et al*.,2015

Figures

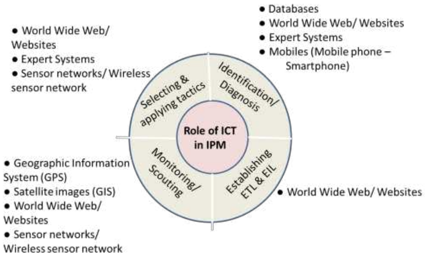

Fig.1. Use of different ICT tools in IPM

Ten years of dubas bug control by using biorational insecticides in Yemen

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Abstract

Dubas bug *Ommatissus lybicus* Bergevin represents the most important economic pest on date palm in Yemen, especially in the Eastern Coastal Area. The evaluation of eco-friendly botanical and biorational insecticides was started in 2004-2006 showed a promising results when using Neem powder and Neem water extract against sucking insects like white fly, thrips and black watermelon bug. The positive preliminary results encouraged us using them against Dubas bug, therefore set of trials have been conducted implementing biorational insecticides in 2007-2008, 2008-2009 and 2009-2010 seasons in spring generation in Wadi Daowan. Neem seed powder extract at a rate of 100g/l water and the methanolic extract of *Cleome droserifolia* Delil at a rate of 3ml/l water were applied compared with a Decis 2.5 EC and water spray. The results of three seasons confirms that all treatments reduced significantly the nymphs population after treatment, however the Neem extract showed a high efficacy on Dubas bugs. In 2012-2013 a formulation of Biorational insecticide Fytomax N (Azadirachtin 1%) was used at a rate of 3ml/litre of water in spring and in autumn-generation at Hadramout coast. Comparisons have been made with Dimethoate 40 EC, and Desirin 250EC (Deltamethrin). The results revealed that the efficacy of Fytomax N to control nymph and adult stages were extremely promising (92.42, 94.0%) and (94.7,93.74%) one week and two weeks after application respectively. In April 2014, another formulated biorational product extracted from the plant *Sophora flavescens,* Matrixine 2.4 was sprayed at a rate of 2.5ml/l water beside Fytomax N1%. Comparisons have been made with chemical insecticides, Desirin250EC (Deltamethrin) and Lambdachem 5%EC(Lamba-cyhalothrin). One day, one week and two weeks after application revealed that the control of nymph and adults, by Matrixine2.4was (93.5,91.6%), (96.5,87.8%), (97.5,90.8%), while that of Fytomax N was (88.5,92.0%), (98.5,63.8%), (95.0,66.0%) respectively. There were no significant differences observed between Matrixine and the chemical insecticides. This outstanding performance encourage us to recommend inclusion of Matrixine2.4 in Dubas bug National campaign control in Yemen as a green biorational solution.

Biological control of root rot, wilt diseases complex in offshoot date palm and improvement of growth parameters in New Valley Governorate, Egypt

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Abstract

Root Rot and wilt disease complex was detected in different date palm offshoots in nurseries and new orchards in New Valley Governorate. Pathogenicity tests showed that *Fusarium oxysporum, F. solani* and *F. moniliforme* were pathogenic to date palm offshoots (cv. Saidy) but they differed in their pathogenic capabilities. The effect of *Bacillus subtilis* (BSM1), *B. megaterium* (BMM5), *B. cereus* (BCM8), *Trichoderma viride* (TVM2), *T. harzianum* (THM4) as bioagents against root rot/ wilt disease complex of date palm offshoots under natural infection in nursery cultivated in two location (El-Kharga and El-Dakhla) were studied. The obtained data indicated that all treatments reduced significantly disease severity compared with untreated offshoots (control) in both locations. *Bacillus megaterium* and *T. viride* recorded the highest protection against disease severity, while *B. cereus* and *T. harzianum* gave the lowest ones in this respect. Under laboratory conditions, all bio-agents inhibited growth of the pathogenic fungi with different percentages. *Bacillus megaterium* and *B. subtilis* were recorded the highest percentage of inhibition growth, while *T. harzianum* gave the lowest ones. On the other hand, all treatments significantly improved growth parameters of date palm offshoots *viz*. offshoot height, number of leaves offshoot^{-1}, leaflet number leaf^{-1}, nick leaf thickness in both locations. *Bacillus megaterium* and *T. viride* recorded the highest all growth parameters whether in El-Kharga or El-Dakhla, while *B. cereus* and *T. harzianum* gave the lowest ones.

On the other hand, all bio-control agents significantly increased chlorophyll a, b and carotenoids in leaf date palm offshoots in both locations. *Bacillus megaterium* and *T. viride* recoded the highest contents of chlorophyll a, b and carotenoids. Also, all treatments increase leaf contents from nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) and Chlorophyll a, b and carotenoid contents in leaves compared with control in both excremental locations. *Bacillus megaterium* recorded the highest levels of N, P, Ca contents in offshoot leaves, while *B. cereus* increased offshoot leaf contents from K, Na and Mg in both locations.

Key words: Biological control agents (BCAs), Date palm offshoots, Growth parameters, Root rot and wilt diseases, Mineral contents

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the major fruit trees in Egypt (El-Assar *et al*., 2005). Date fruit consumption is an important source of supplying mineral and vitamin elements in a balanced nutrition regime (Al-Shahib and Marshall, 2003). Date palm trees and offshoots are attacked by several soil borne pathogenic fungi at different regions around the world causing severe losses and deterioration of trees and new offshoots. *Fusarium* *oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *F. semitectium*, *Rhizoctonia solani* has been reported in different countries to cause root rot and wilt diseases in young and adult date palm trees (El-Morsi *et al*., 2009 and Maitlo *et al*., 2013).

Agricultural practices for management of soil borne pathogens in the field includes cultural practices, crop rotation, fungicide applications, methyl bromide fumigation, soil solarization and use of resistant or tolerant varieties. At present, no single method provides adequate control of soil borne diseases (Hausbek and Lamour, 2004). Using of chemicals to control soil borne pathogens causes several negative effects, such as: i) development of pathogen resistance, ii) hazards to humans, iii) damage to beneficial organisms and iv) environmental pollution. Moreover, many chemicals will be banned in near future. However, for sustainable production, pathogens still need to be controlled in order to ensure healthy plant establishment and growth (Gerhardson, 2002). Therefore, developments of various beneficial micro-organisms or biological control agents' methods are urgently needed in order to provide an alternative to chemical control. Among different biological approaches, use of the microbial antagonists like yeasts, fungi and bacteria could be promised, effectively, safely and eco-friendly in controlling many of soil borne pathogens (Gravel *et al*., 2004). Many biological control agents such as *Trichoderma* spp. and *Bacillus* spp. could be effectively used in suppressing diseases caused by *Fusarium* spp. as reported by many workers (Abdel- Monaim, 2010 and Perveen and Bokhari, 2012). Modes of action for beneficial micro-organisms include direct parasitism of plant pathogens, competition for space or nutrients, or production of antibiotics, enzymes or plant hormones (Lugtenberg *et al*., 2003). This led to promote plant growth during the growing season as reported by Wahyudi *et al*. (2011). However, up to date, only a few antagonist microorganisms have been identified as potential, effective biological control agents (BCAs) against soil borne pathogens (Spadaro and Gullino, 2005). Also, these bioagents increased significantly due to root growth and increased of plant growth in date palm and other many crops (Abdel-Monaim, 2010 and Perveen and Bokhari, 2012). On the other hand, PGPR as biofertilization played an important role in plant nutritional requirements; since biofertilizers were reported to enhance crop productivity through improving plant nutrition, enhancement of nutrients availability, nitrogen fixation, phosphate solubilization, increased leaf contents of chlorophyll, carotenoids and plant hormone production (Lazarovits and Nowak, 1997 and Yaso *et al*., 2007 and Isfahani and Beshara, 2012).

The main objective of the present study was to evaluate the effectiveness of isolated micro-organisms from soil under New Valley governorate conditions as biological control agents (BCAs) against the incidence of root rot and wilt diseases caused by *F. oxysporum, F. solani* and *F. moniliforme* and its impact on vegetative growth of date palm offshoots cv. Saidy under field conditions. In addition, its effect on chlorophylls, carotenoids and mineral contents in offshoot leaves.

MATERIALS AND METHODS

Isolation of the causal fungi:

Roots samples from naturally infected date palm offshoots (cv. Saidy) were collected from different locations of New Valley governorate during growing season 2015. Infected roots were washed several times with tap water to remove the attached soil particles. The samples were then cut into small pieces, rinsed several times in sterilized distilled water, disinfected by 0.1% sodium hypochlorite solution for one minute, followed by washing in three changes of sterilized water and dried between folds of sterilized filter paper. The sterilized fragments were aseptically transferred to Petri dishes containing 20 ml of potato dextrose agar (PDA) medium, and incubated at 25 °C for 5 days. The isolated fungi were purified using the single spore technique and / or the hyphal tip method. The isolates were identified based on published descriptions (Nelson *et al*., 1983 and Booth, 1985) of morphological and cultural characteristics mycelium, conidiophores, conidia and colony morphology.

Pathogenicity tests:

The pathogenic capability of the isolated fungi was carried out under greenhouse conditions in El-Kharga Agric. Res. Station, New Valley, Egypt. Date palm seeds (cv. Saidy) were treated with dry heat at 45 °C for 2 hours to activate seed germination then planting in Plastic pots (30 cm in diameter) were filled with autoclaved soil (2 kg pot^{-1}) at rate one seed pot^{-1}. After 6 months from planting, pot were inoculated with the pathogenic fungal by using homogenized culture technique (Muthomi *et al*., 2007). Disks taken from 1- wk-old culture of isolate of the tested fungi were inoculated in 75 mL of potato dextrose broth (PDB) in a 250 mL flask and incubated at 25 ± 1ºC. The obtained fungal cells were collected on Whatman No. 1 filter paper, rinsed with sterile distilled water, placed in a waring blender with a small amount of sterile water, and blended for 2 min at high speed. Sterile distilled water was then added to each inoculum suspension to give a final concentration of 10^6 colony forming units (CFU/mL) that was used for soil infestation. Five pots were used as a replicates for each fungal isolate along with check treatment (un- infested soil). The pots were irrigated as needed.

The severity of root rot/wilt complex was determined after 90 days (Abdou *et al*., 2001) using a rating scale of 0-5 on the basis of root discoloration or leaf yellowing: 0: no root discoloration or leaf yellowing; 1: 1-25% root discoloration or one leaf yellowed; 2: 26-50% root discoloration or more than one leaf yellowed; 3: 51-75% root discoloration plus one leaf wilted; 4: up to 76% root discoloration or more than one leaf wilted; and 5: completely dead plants. For each replicate a disease severity index (DSI) similar to that described previously (Liu *et al*., 1995) was calculated as follows:

$$\mathrm{DSI}= \frac{\sum \mathrm{d}}{\mathrm{d\ max} \times \mathrm{n}} \times 100$$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

***In vitro* Studies:**

The used antagonistic organisms were *Bacillus subtilis* (BCM1), *B. megaterium* (BMM5), *B. cereus* (BCM8), *Trichoderma viride* (TVM2) and *T. harzianum* (THM4). These isolates provided by Dr. Montaser, F. Abdel-Monaim, Plant Pathol. Res. Inst., Agric. Res. Center (Abdel-Monaim, 2010). *Trichoderma* isolates and the tested pathogenic fungi (*F. oxysporum*, *F. solani* and *F. moniliforme*) were cultured on PDA medium for 7 days at 25±1 ºC. A disc (0.7 cm diameter) of the antagonistic fungal colony was cut and placed opposite to the colony of the pathogenic fungal isolates on PDA medium. On the other hand, *Bacillus* isolates were streaked at opposite ends of PDA plates near edge and incubated at 25±1 ºC for 24 hr. Then a mycelial disc (0.7 cm) of the tested fungi was placed in the center of each plate. For control treatment, the agar plug of only pathogen isolates was placed on PDA plates. The inoculated plates incubated at 25±1 ºC until colony of control grew to full plate. At this point, colony diameter was measured using ruler. Percentage of growth inhibition of pathogen was calculated using the formula below:

$$\% \text{ Inhibition} = (A\text{-}B)/A \times 100$$

Where: A = Colony diameter of pathogen in control, B = Colony diameter in treated plates

***In vivo* studies:**

Preparation of formulated antagonistic fungi and bacteria:

Inoculum of antagonistic bacteria *viz*. *B. subtilis* (BSM1), *B. megaterium* (BSM5) and *B. cereus* (BCM8) were produced in 100 ml of potato dextrose broth (PDB) medium (pH7) in 250 ml conical flasks, on an orbital shaker at 125 rpm and 28±1°C for 3 days. Bacterial cells were harvested by centrifugation (10,000 × *g* for 20 min) and washed twice with sterile 0.1 M MgSO4. Bacterial concentration in the suspension was adjusted to proximately 5×10^8 cells per ml by measuring absorbance at 600 nm (*A*600) in a spectrophotometer and using standard curves for each bacterial isolate. While, inoculum of the antagonistic fungi *viz*. *T. viride* (TVM2) and *T. harzianum* (THM4) was prepared by culturing on 50.0 mL potato dextrose broth (PDB) medium (ph5) in 250 mL Erlenmeyer flasks for 10 days at 25±2°C following washing and blending in sterilized water. Colonies forming units (cfu) were adjusted to 10^6 cfu/ mL using haemocytometer slide.

Effect of biological control agents (BCAs) on root rot / wilt and growth parameters under field conditions:

Field experiments was carried out at New Valley Agric. Res. Station Farm and Directorate of Agriculture in El-Dakhla, New Valley governorate during 2016 season, to evaluate the efficiency of the tested biological control agents (*Bacillus subtilis, B. megaterium B. cereus*, *Trichoderma viride* and *T. harzianum*) individually for controlling root rot and wilt diseases of date palm offshoots (cv. Saidy) as well as its effect on growth parameters. The chosen field test area was naturally infested with the causal organisms of root rot and wilt pathogens. The experimental design was a complete randomized block with four replicates. The experimental unit area was 2 m2 (1 × 2 m). Each unit included 2 date palm offshoots (3-years-old). Soil of the planting with offshoots were drenched three time at 15 – day intervals with bio- control agents inocula prepared as above at rate 3 L per offshoot. Untreated soil was used for control. The disease severity was assessed for each treatment after 6 months from the least of application treatments. After the end of this experiment the following estimation were take: root numbers offshoot^{-1}, offshoot height (cm), number of leaves offshoot $^{-1}$, leaflet numbers leaf $^{-1}$ and Leaf thickness (cm)

Estimation of chlorophyll and carotenoid contents.

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). 500 mg of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001- Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon (1949) and expressed in milligram per gram fresh weight.

Chlorophyll 'a' (mg/ml) = (0.0127) × (A.663) – (0.00269) × (A.645)

Chlorophyll 'b' (mg/ml) = (0.0229) × (A.645) – (0.00468) × (A.663)

Carotenoid content was estimated using the formula of **Kirk and Allen (1965)** and expressed in milligrams per gram fresh weight.
Carotenoid (mg/g) = A.480 + (0.114 × A.663 – 0.638 × A.645)

Leaf mineral contents:

The leaf samples were washed with tap water, rinsed twice in distilled water and air dried in an oven at 70°C. The dried leaves were ground and digested with H_2O_2 and H_2SO_4 according to Evanhuis and De Waard (1980). Suitable aliquots were taken for the determination of the mineral content. Nitrogen was determined by the Kjeldahl method (AOAC, 1995). Phosphorus was determined by the ascorbic acid method according to Murphy and Riley (1962). Potassium and sodium were determined with a flame photometer. The Ca, Mg contents were measured using atomic absorption spectrophotometer. The concentrations of N, P, K, Ca, Na and Mg were expressed as percentages.

Statistical analysis:

All experiments were performed twice. Analyses of variance were carried out using MSTAT-C, 1991 program version 2.10. Least significant difference (LSD) was employed to test for significant difference between treatments at $P \leq 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSIONS

Isolation, Identification of the causal organism (S) and pathogenicity tests:

Five Fusarium species were isolated from date palm offshoots showing root rot and wilt symptoms. These species were identified as *Fusarium oxysporum*, *F. equiseti*, *F. solani*, *F. Semitectium* and *F. moniliforme.* The pathogenicity tests indicate that all the tested fungi significantly caused root rot and wilt diseases in date palm offshoots var. Saidy (Fig. 1). *Fusarium oxysporum* was the most pathogenic fungi as they recorded percentage root rot/wilt severity (89.26%) followed by *F. solani* and *F. moniliforme*, where caused 82.18% and 73.26% disease severity, respectively. On the contrary, *F. equiseti* and *F. semitectium* were the least pathogenic ones recording the lowest percentages of these criteria.These results are in harmony with those reported by other researchers (El-Morsi *et al*., 2009 and Maitlo *et al*., 2013).

Effect of biological control agents (BCAs) on root rot and wilt severity under field conditions:

Data present in Fig. 2 showed that all tested biological control agents (BCAs) significantly decreased root rot and wilt disease complex under naturally infection in field cultivated in El-Kharga and El-Dakhla. Efficiency of the tested BCAs for controlling this disease on date palm offshoots var. Saidy was varied. However, *B. megaterium* and *T. viride* was the most effective BCAs for decreasing root rot and wilt severity, being 13.47,18.96 and 18.83, 25.36% compared with 75.38, 86.36% disease severity in control in both locations, respectively. Meanwhile, *B. cereus* and *T. harzianum* gave the lowest effect ones while recorded 36.70, 40.80 and 39.36, 43.36 % disease severity, respectively.

Control of root rot and wilt diseases in date palm offshoots depends mainly on fungicides application (El-Morsy *et al*., 2012). Meanwhile, fungicides always undesirable due to high coast, probability of development of resistant strains and potential hazards to the environment. An option for reducing pollution caused by the use of synthetic agrochemical in date palm offshoots disease management is bio-control by using of antagonist microorganisms belonging to the *Bacillus* spp. and/or *Trichoderma* spp., because they are considered the most efficient for their inhibitory properties (El-Mohamedy and Ahmed, 2009), stimulation of plant growth (Wahyudi *et al*., 2011).

***In vitro* screening inhibitory effect of biological control agents (BCAs):**

Bacillus subtilis, B. megaterium, T. viride and *T. harzianum,* strains were evaluated for antagonistic effect against *F. oxysporum, F. solani and F. moniliforme* on Petri dishes containing PDA medium. Fig. 3 show that the bio-agent strains succeeded in reducing the radial growth of the tested pathogenic fungi. *B. megaterium* and *B. subtilis* was active more than the other tested bioagents for reducing the radial growth of the tested pathogenic fungi. The percent inhibition of radial growth of tested fungi *viz*., *F. oxysporum* (68.49 and 55.26%), *F. solani (*77.36 and 70.14%), and *F. moniliforme* (52.08%) were reduced by *B. megaterium and B. subtilis,* respectively. On contrary, *T. harzianum* gave the lowest ones in this respect. Generally, *Bacillus* strains more than *Trichoderma* strains inhibition of redial growth of all the tested fungi and the greatest reduction occurring in *F. solani* followed by *F. oxysporum* , while *F. moniliforme* less affected ones.

Effect of bio-control control agents on growth parameters:

Data present in Table 1 and 2 showed that all tested biological control agents (BCAs) significantly increased all growth parameters *viz.* root numbers offshoot^{-1}, offshoot height (cm), number of leaves offshoot $^{-1}$, leaflet number leaf^{-1} and nick leaf thickness of date palm offshoots (var. Saidy) compared with control whether in El-kharga or El-Dakhla. *B. megaterium* and *T. viride* recorded the highest increased of all growth parameters, where increased of root numbers offshoot $^{-1}$ from 4.33, 3.33 in control to 23.67, 20.33 and 21.00, 18.67, offshoot height from 110.59, 106.00 in control to 235.33, 218.69 and 222.32, 230.14 cm and number of leaves offshoot $^{-1}$ from 1.17, 1.36 to 5.83,6.05 and 5.17, 6.36 in both locations, respectively. Also, both treatments increased leaflet number leaf^{-1} from 26.25, 28.36 in control to 121, 125.36 and 100.17, 105.36, leaf thickness from 0.65, 0.72 in control to 1.52, 1.63 and 1.42, 1.36 cm in both locations, respectively. On the other hand, *B. cereus* and *T. harzianum* gave the lowest ones in all growth parameters. Our study showed that date palm offshoots treated with biological control agents (BCAs) caused higher reduction in root rot and wilt severity compared to the untreated control plants (Mogle and Mane, 2010, Nihorimbere *et al*., 2010) and promote the growth of a wide range of plants (Wahyudi *et al*., 2011 and Al-Rajhi, 2013). Biological control agents (BCAs) such as *Bacillus* and *Trichoderma* species help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content (Al-Taweil *et al*., 2009). Antagonistic microorganisms retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients (Baset *et al*., 2010). *Bacillus* and *Trichoderma* also have been known to produce compounds which promote plant growth directly or indirectly *viz*., hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorous, *Trichoderma* spp. could elucidate to produce trichotoxins promoting plant and antifungal activity (Shobha and Kumudin, 2012).

The mechanism of antagonistic microorganisms action on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Zaghloul *et al*., 2007), produce siderophores and hydrogen cyanide (Soleimani *et al*., 2005), production of secondary metabolites such as Phenazine -1-Carboxilic acid (PCA), 2,4-Pyrrolnitrin, Oomycin (Knudsen, 1995) and production of antibiotics (Ehteshamul-Haque and Ghaffar, 1993).

Photosynthetic pigment contents

Data present in Tables (3 and 4) indicated that all bio-agents significantly increased chlorophyll a and b and carotenoid contents in date palm offshoots leaves compared with control in both experimental locations (El-Kharga and El-Dakhla). *Trichoderma viride* and *B. megaterium* recorded the highest chlorophyll a and b and carotenoids contents in leaves in both locations, while *B. cereus* gave the lowest increased of chlorophyll a and b and carotenoids contents in leaves in both locations. These results were same with those reported by (Isfahani and Beshara, 2012). Amro *et al* (2014) on" Hayany" date palm indicated that using EM at 90 ml / palm/ year enriched with potassium sulphate at 1.5kg/palm/year treatment as soil application enhance leaf chlorophyll content, retained fruit percentage, yield, fruit quality and leaf minerals content.

Mineral contents

Data present in Tables 5 and 6 shows that all treatments significantly increased mineral contents in date palm offshoots *viz.* N, P, K, Ca, Na and Mg compared with control in both locations (El-Kharga and El-Dakhla). *B. megaterium* recorded the highest levels of N, P and Ca in date palm offshoots, while *B. cereus* recorded the highest levels of K, Na and Mg contents in date palm offshoots in both locations.

Biofertilization methods played an important role in plant nutritional requirements; since biofertilizers were reported to enhance crop productivity through improving plant nutrition, enhancement of nutrients availability, nitrogen fixation, phosphate solubilization, and plant hormone production (Lazarovits and Nowak, 1997). Abd –Elmoniem and Radwan (2003) reported that, Micro-organisms in bio-fertilizers maximize the availability of nutrients in the soil and improve their uptake and utilization.

CONCLUSION

Results of the present study could suggest that soil drench with biological control agents (BCAs) can be used as a safe control measure of the disease on date palm offshoot and as a stimulant of vegetative growth parameters.

REFERENCES

Abd EL-Moniem, Eman A.A. and Radwan S.M.A. (2003). Response of Willliams banana plants to bio fertilization in relation to growth, productivity and fruit quality. Arab Univ. J. Agric. Sci,. Ain Shams Univ., Cairo,11 (2):751- 763

Abdel-Monaim, M.F. (2010). Integrated management of damping- off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. Egypt. J. Phytopathol., 38(1-2): 45-61.

Abdou E., Abd-Alla H.M. and Galal A.A. (2001). Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assuit J Agric Sci., 32:135–152.

Al-Rajhi A.M.H. (2013). Impact of biofertilizer *Trichoderma harzianum* Rifai and the biomarker changes in *Eruca sativa* L. plant grown in metal-polluted soils. World Appl. Sci. J. 22 (2): 171-180.

Al-Shahib W. and Marshall R.J. (2003). The fruit of the date palm: it's possible use as the best food for the future? Inter. J. of Food Sci. and Nutrition, 54 (4): 247-259.

Al-Taweil H.I., Osman M.B., Hamid A.A. and Wan Yussof W.M. (2009). Development of microbial inoculants and the impact of soil application on rice seedlings growth. Am. J. Agric. Biol. Sci., 4:79-82.

Amro, S.M., El- Sayed Omima M. and Osama H.M. (2014). Effect of effective microorganisms (EM) and potassium sulphate on productivity and fruit quality of "Hayany" date palm grown under salinity stress. J. of Agric. and Veterinary Sci., 78(7): 90-99.

AOAC. (1995). Association of Official Agricultural Chemists, Official Methods of Analysis, 15th ed. A.O.A.C., Washington, DC.

Arnon D.J. (1949). Copper enzymes in isolated chloroplast. polyphenol oxidase in Beta Vulgaris. Plant Physiol, 24:1-15.

Baset M.M.A., Shamsuddin Z.H., Wahab Z. and Marziah M. (2010). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Aust. J. Crop Sci., 4: 85-90.

Booth C. (1985). The genus Fusarium. Surrey: Commonwealth Mycological Institute.

Ehteshamul-Haque S. and Ghaffar A. (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. J. Phytopath., 138:157-163.

El-Assar A.M., Krueger R.R., Devanand P.S. and Chao C.C.T. (2005). Genetic analysis of Egyptian date (*Phoenix Dactylifera* L.) accessions using AFLP markers. Genet Resour Crop Ev 52: 601–607

El-Mohamedy R.S.R. and Ahmed M.A. (2009). Effect of biofertilizers and humic acid on control of dry root rot disease and improvement yield quality of mandarin (*Citrus reticulate* Blanco). Res. J. of Agric. and Biological Sci., 5(2): 127-137.

El-Morsi M.E.A., Abo Rehab M.E.A. and El-Morsy S.A. (2012). Survey and control trials of root rot/wilt of date palm offshoots in New Valley Governorate. Egypt. J. Agric. Res., 90 (4): 1403-1414.

El-Morsi M.E.A., Kamhawy M.A.M. and Sallam M.A.A. (2009). Effectiveness of some organic compounds in controlling pathogenic fungi associated with roots of date palm offshoots in New Valley Governorate, Egypt. Assiut J. of Agric. Sci., The 3 rd Con. of Young Scientists, Fac. of Agric., April, 28, 40: 137-150.

Evanhuis B. and De Waard P.W. (1980). Principles and practices in plant analysis. FAO Soils Bull. 38: 152–163.

Gerhardson B. (2002). Biological substitutes for pesticides. Trends Biotechnol. 20:338-343.

Gomez K.A. and Gomez A.A. (1984). Statistical Procedures For Agricultural Research. New York: Wiley Interscience Publication; pp. 678.

Gravel V., Martinez C., Antoun H. and Tweddell R.J. (2004). Evaluation of antagonistic microorganisms as biological control agents (BCAs) of root rot (*Pythium ultimum*) of greenhouse tomatoes in rock wool. Can. J. Plant Pathol. 26: 152-159.

Hausbek M.K. and Lamour K.H. (2004). *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Disease 88, 1292–1303.

Isfahani F.M. and Besharati H. (2012). Effect of biofertilizers on yield and yield components of cucumber. J Biol Earth Sci, 2(2): 83-92.

Kirk J.T.O. and Allen R.L. (1965). Dependence of chloroplast pigments synthesis on protein synthetic effects of acitilione. Biochem. Biophys. Res. Cann., 27: 523-530.

Knudsen I.M., Hockenhull B.J. and Jensen D.F. (1995). Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. Plant Pathol. 44: 467-477.

Lazarovits G. and Nowak J. (1997). Rhizobacteria for improvement of plant growth and establishment. HortScience 32(2): 188-192.

Liu L., Kloepper J.W. and Tuzun S. (1995). Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology, 85:695-8.

Lugtenberg B.J.J., Bloemberg G.V., Woeng C.A. and Thomas F.C. (2003). Phenazines and their role in bio-control by *Pseudomonas* bacteria. New Phytologist, 153: 503-523.

Maitlo W.A., Markhand G.S., Abul-Soad A., Lodhi A.M. and Jatoi M.A. (2013). Chemical control of Sudden decline disease of date palm (*Phoenix Dactylifera* L.) in Sindh, Pakistan. Pak. J. Bot., 45(S1): 7-11.

Mogle U.P. and Mane R.Y. (2010). Antagonistic effect of bio-fertilizers against seed born mycoflora of tomato (*Lycopersicun esculentum*). Res. J. of Agri. Sci., 1(3): 255-258.

MSTAT-C, (1991). A Software Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State University, pp. 400.

Murphy, J., Riley, J.P. (1962). A modified single solution method for the determination of phosphorus in natural water. Anal. Chin. Acta 27, 31–36.

Muthomi J.W., Otieno P.E., Chemining'wa G.N., Nderitu J.H. and Wagacha J.M. (2007). Effect of legume root rot pathogens and fungicide seed treatment on nodulation and biomass accumulation. J Biol Sci. 7:1163-70.

Nelson E.P., Toussoun A.T. and Marasas O.F.W. (1983). *Fusarium* species. An Illustrated Manual for Identification. The Pennsylvania state Univ. Press. P. 191.

Nihorimbere V., Ongena M., Cawoy H., Brostaux Y., Kakana P., Jourdan E. and Thonart P. (2010). Beneficial effects of *Bacillus subtilis* on field-grown tomato in Burundi:

Reduction of local *Fusarium* disease and growth promotion. Afr. J. of Microbiol. Res., 4 (11), 1135-1142.

Perveen K. and Bokhar N.A. (2012). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. Afr. J. of Microbiology Res., 6(13): 3348-3353

Shobha G. and Kumudini B.S. (2012). Antagonistic effect of the newly isolated PGPR *Bacillus* spp. on *Fusarium oxysporum*. Int. J. Appl. Sci. Eng. Res. 1, 463-474.

Soleimani, M. J., Shamsbakhsh M., Taghavi M. and Kazemi S. H. (2005). Biological control of stem and root-rot of wheat caused by *Biopolaris* spp by using antagonistic bacteria, Fluorescent Pseudomonas and *Bacillus* spp. J. Biol. Sci. 5, 347-353.

Spadaro D. and Gullino M.L. (2005). Improving the efficacy of biological control agents (BCAs) against soil borne pathogens. Crop Protection, 24: 601-613.

Wahyudi A.T., Astuti R.I. and Giyanto (2011). Screening of *Pseudomonas* sp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. Am. J. Agric. Biol. Sci., 6: 134-141.

Yaso I.A., Abdel-Razzak H.S. and Wahb-Allah M.A. (2007). Influence of bio-fertilizer and mineral nitrogen on onion growth, yield and quality under calcareous soil conditions J. Agric. & Env. Sci. Alex. Univ., Egypt, 6 (1): 245-264.

Zaghloul R.A., Hanafy, E.A., Neweigy N.A., Khalifa N.A. (2007). Application of biofertilization and biological control for tomato production. 12th Con. of Microbiol.; Cairo, Egypt, (18-22) March, 198-212.

Tables

Table (1). Effect of biological control agents (BCAs) on growth parameters of date palm offshoots Var. Saidy under field conditions in El-Kharga.

Treatments	Root numbers offshoot^{-1}	offshoot height (cm)	Number of leaves offshoot^{-1}	Leaflet number leaf^{-1}	Nick leaf thickness
Bacillus subtilis (BSM1)	16.33 c	198.19 c	4.33 c	88 c	1.22 c
B. megaterium (BMM5)	23.67 a	235.33 a	5.83 a	121 a	1.52 a
B. cereus (BCM8)	12.00 d	172.58 d	3.67 cd	68.5 d	1.09 d
Trichoderma viride (TVM2)	21.00 b	222.32 b	5.17 b	100.17 b	1.42 b
T. harzianum (THM4)	10.67 e	165.36 e	3.00 d	62.67 e	1.18 cd
Control	4.33 f	110.59 f	1.17 e	26.25 f	0.65 e

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Table (2). Effect of biological control agents (BCAs) on growth parameters of date palm offshoots Var. Saidy under field conditions in El-Dakhla.

Treatments	Root numbers offshoot^{-1}	offshoot height (cm)	Number of leaves offshoot^{-1}	Leaflet number leaf^{-1}	Nick leaf thickness
Bacillus subtilis (BSM1)	17.00 c	205.47 c	5.04 b	82.47 c	1.13 c
B. megaterium (BMM5)	20.33 a	218.69 b	6.05 a	125.36 a	1.63 a
B. cereus (BCM8)	10.67 d	163.34 e	4.25 c	70.25 d	1.02 d
Trichoderma viride (TVM2)	18.67 b	230.14 a	6.36 a	105.36 b	1.36 b
T. harzianum (THM4)	9.67 d	149.67 d	2.55 d	66.35 e	1.09 cd
Control	3.33 e	106 f	1.36 e	28.36 f	0.72 e

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Table (3). Effect of biological control agents (BCAs) on chlorophylls a, b and carotenoids (mg/g f.w.) of date palm offshoots Var. Saidy under field conditions in El-Kharga.

Treatments	Chlorophyll-a (mg/g fresh weight)	Chlorophyll-b (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
Bacillus subtilis (BSM1)	0.89b	0.77b	0.68c
B. megaterium (BMM5)	1.09a	0.92a	0.77b
B. cereus (BCM8)	0.79b	0.65c	0.58d
Trichoderma viride (TVM2)	1.12a	0.99a	0.89a
T. harzianum (THM4)	0.85b	0.75b	0.65c
Control	0.62c	0.49d	0.36e

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Table (4). Effect of biological control agents (BCAs) on chlorophylls a, b and carotenoids (mg/g f.w.) of date palm offshoots Var. Saidy under field conditions in El-Dakhla.

Treatments	Chlorophyll-a (mg/g fresh weight)	Chlorophyll-b (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
Bacillus subtilis (BSM1)	0.96b	0.85b	0.75bc
B. megaterium (BMM5)	1.12a	0.96a	0.82c
B. cereus (BCM8)	0.85c	0.72c	0.63d
Trichoderma viride (TVM2)	1.15a	0.95a	0.92a
T. harzianum (THM4)	0.92bc	0.78bc	0.69cd
Control	0.68d	0.52d	0.42e

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Table (5). Effect of biological control agents (BCAs) on nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots var. Saidy under field conditions in El-Kharga.

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)
Bacillus subtilis (BSM1)	1.16b	1.36b	0.42c	0.16c	0.019abc	0.61b
B. megaterium (BMM5)	1.42a	1.97a	0.49bc	0.22b	0.029a	0.65b
B. cereus (BCM8)	1.09b	1.25b	0.58a	0.28a	0.015abc	0.75a
Trichoderma viride (TVM2)	1.36a	1.85a	0.53ab	0.23b	0.025ab	0.68b
T. harzianum (THM4)	1.16b	1.12c	0.42c	0.13cd	0.011bc	0.39c
Control	0.76c	0.86d	0.31d	0.09d	0.009c	0.31c

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Table (6). Effect of biological control agents (BCAs) on nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots var. Saidy under field conditions in El-Dakhla.

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)
Bacillus subtilis (BSM1)	1.25c	1.42c	0.39de	0.24ab	0.020abc	0.69b
B. megaterium (BMM5)	1.65a	1.95a	0.48bc	0.23b	0.035a	0.70b
B. cereus (BCM8)	1.02d	1.21d	0.58a	0.30a	0.019bc	0.84a
Trichoderma viride (TVM2)	1.45b	1.77b	0.52ab	0.25ab	0.028ab	0.75b
T. harzianum (THM4)	1.21c	1.24d	0.44cd	0.14c	0.012c	0.42c
Control	0.81e	0.90e	0.34e	0.11c	0.010c	0.32d

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P≤ 0.05).

Figures

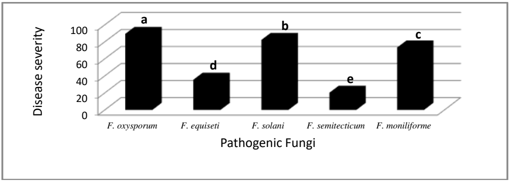

Fig 1. Pathogenic ability of five Fusarium species on root rot and wilt severity of date palm offshoots (var. Saidy) under greenhouse conditions. Different letters indicate significant differences among pathogenic fungi within the same column according to least significant difference test (p≤0.05).

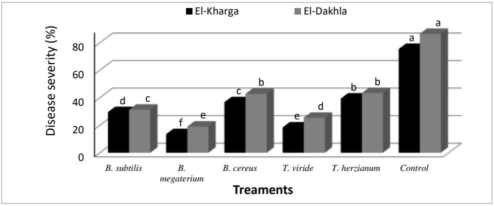

Fig 2. Effect of biological control agents (BCAs) on disease severity of root rot/ wilt disease of date palm offshoots var. Saidy under field conditions cultivated in El-Kharga and El-Dakhla. Different letters indicate significant differences among treatments within the same column according to least significant difference test (p≤0.05).

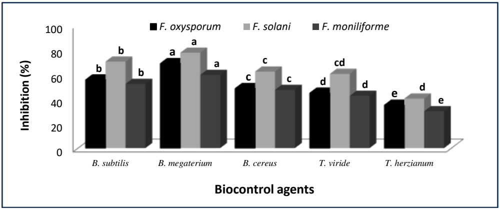

Fig. 3. Inhibitory effect of some biological control agents (BCAs) isolates on mycelial growth of date palm offshoots pathogenic fungi. Different letters indicate significant differences among biological control agents (BCAs) within the same colour column according to least significant difference test ($p \leq 0.05$).

Assessment of damage and biology of the major storage pests of dates (*Phoenix Dactylifera* L.)

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Abstract

Insect infestation and damage caused by insect feeding on the dates is one of the primary causes of postharvest losses in quality and quantity. The extent of damage was assessed in ten different cultivars of dates, which revealed increased intensity of infestation with increase in duration of storage period. Maximum damage of 61 per cent was recorded in the cultivar Deglet Nour after two months of storage under laboratory conditions. The fruits become highly unsuitable for consumption producing foul odour, change in colour and flavour. The major pests infesting stored dates were, *Tribolium castaneum* (Herbst.), *Oryzaephilus surinamensis* (Linn.) and *Carpophilus hemipterus* (Fab.). With the objective to manage these three pests, studies were undertaken on their biology to understand the weakest link in their life stages, which revealed the total development period from egg to adult as 33 to 48 days in *Tribolium castaneum*, 39 to 54 days in *Oryzaephilus surinamensis* and 35 to 45 days in *Carpophilus hemipterus*.

Keywords: Insect pests, dates, *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Carpophilus hemipterus,* damage, biology.z

INTRODUCTION

Phoenix dactylifera L., commonly known as the date palm is a primeval flowering plant species in the family Arecaceae and has been cultivated for its edible fruit. Dates is one of the major food commodity which provides a rich source of carbohydrates, dietary fibres, certain essential vitamins and minerals. Dates contain high amounts of essential nutrients: minerals (Mg, Ca, Fe, K), carbohydrates (total sugars 44–88 %, glucose and fructose 65–80 % of dry weight) vitamins (niacin, B1, B2), dietary fibers (6.4–11.5 %), fatty acids and proteins. They could play an important role in emergency food relief programs. Certain dates cultivars are considered to have several medicinal qualities *viz.,* antifungal, antibacterial, antiulcer, immuno-modulatory and antitumor properties. Also, some date cultivars have antioxidant activity due to phenolic compounds. The dates are an excellent source of dietary fiber and contain considerable amounts of minerals, lipids and protein (Baliga *et al.,* 2011).

Efficient utilization of raw dates and date seeds is expected to generate a number of new products by bioprocessing, especially through the exploration of these technologies on commercial scale by the pharmaceutical and food industries (Chandrasekaran and Bahkali, 2013). Date seeds are also used in animal feed due to their high protein, fat and dietary fiber contents (Besbes *et al*., 2004a,b,c). About 11–18 % of date fruit weight is from the seed, where a recent report suggests that the antioxidant content of date seed oil is comparable to that of olive oil (Abdul Afiq *et al*., 2013). Fresh dates are perishable and are highly susceptible to losses from damage by stored product insects and causes quantity deterioration between harvest and consumption, leading to loss of quantity, quality and market value. Dates may become infested at the processing plant or warehouse, in transit, or at the store.

The earliest report on the insect pests of date palm goes almost one hundred years back when Buxton, (1920) documented the insect pests of date palm in Mesopotamia (present day Iraq). Later, Carpenter and Elmer, (1978) listed 54 species of insect pests and mites on dates and date palms. A comprehensive report from Israel on the arthropod pest complex of date palm and their management lists 16 major and 15 minor insect pests (Blumberg, 2008). Recently, (El-Shafie, 2012), listed 112 species of mites and insects worldwide associated with date palm, including 22 species attacking stored dates. The list of common pests of stored dates is listed in Table 1.

Of the arthropod pests listed above, three are considered major pests damaging dates *viz., Tribolium castaneum* (Herbst.), *Oryzaephilus surinamensis* (Linn.), and *Carpophilus hemipterus* (Fab.). On these grounds, to understand the intensity of damage to manage these three pests, studies were undertaken on assessing damage potential of insect pests in ten different dates cultivars and biology of the three major storage pests of dates.

MATERIALS AND METHODS

Assessment of percent damage of storage pests in dates

Fresh dates of selected ten cultivars (Ajwah, Mazafati, Medjool, Deglet Nour, Jabri, Barakawi, Fardah, Hamraya, Barhee and Khalasah) were purchased from the market. Five hundred gram of the samples were stored in plastic containers with five replications for a period of two months. The extent of damage was assessed by counting the number of infested dates and the total number of dates. The percentage of damage was calculated using the following formula.

$$\text{Per cent Damage} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 100$$

Biology of *Tribolium castaneum*: The red flour beetle, *Tribolium castaneum* was cultured on dates in the cultivar Ajwah under laboratory conditions. Initially 10 red flour beetle adults were collected from infested samples and introduced in petri dishes containing 10 gm of date fruits. The petri dishes were secured with cellophane tape to avoid escape of adults. One week after the release, the adult beetles were removed after egg laying. Studies to determine the duration of different stages *viz*., egg, larva, pupa and adult were carried out in petri dishes containing 10gm cut pieces of dates. Ten replications were maintained for the study. Observations were made on a daily basis for recording the egg, larval and pupal periods.

Biology of *Oryzaephilus surinamensis*: The saw-toothed grain beetle, *Oryzaephilus surinamensis* was cultured on dates in the cultivar Deglet Nour under laboratory conditions. Initially 10 saw toothed grain beetle adults were collected from infested samples and introduced in petri dishes containing 10 gm of date fruits. The petri dishes were secured with cellophane tape to avoid escape of adults. One week after the release, the adult beetles were removed after egg laying. Studies to determine the duration of different stages *viz*., egg, larva, pupa and adult were carried out in petri dishes containing 10 gm cut pieces of dates. Ten replications were maintained for the study. Observations were made on a daily basis for recording the egg, larval and pupal periods.

Biology of *Carpophilus hemipterus*

The dried fruit beetle, *Carpophilus hemipterus* was cultured on dates in the cultivar Mazafati under laboratory conditions. Initially 10 dried fruit beetle adults were collected from infested samples and introduced in petri dishes containing 10 gm of date fruits. The petri dishes were secured with cellophane tape to avoid escape of adults. One week after the release, the adult beetles were removed after egg laying. Studies to determine the duration of different stages *viz*., egg, larva, pupa and adult were carried out in petri dishes containing 10gm cut pieces of dates. Ten replications were maintained for the study. Observations were made on a daily basis for recording the egg, larval and pupal periods.

Statistical analysis

The data on assessment of damage were transformed into arcsine and square root values, respectively before subjecting them to statistical analysis (Gomez and Gomez, 1984). Analysis of variance was done in AGRESS and AGDATA Packages.

RESULTS AND DISCUSSION

Assessment of damage potential

All the ten cultivars were subjected to two months of storage and the extent of damage was assessed statistically (Table 2). Damage symptoms included change in texture, colour, presence of holes due to feeding and presence of powdery coating on the fruits due to feeding. The percentage of damage in selected cultivars of dates ranged from (0 to 35) during the first week with highest damage in cultivar Deglet Nour (35 per cent) followed by Mazafati (31 per cent), Ajwah (30 per cent), Hamraya (30 per cent), Barakawi (27 per cent), Medjool (19 per cent), Fardh(18 per cent), Jabri (11 per cent), Barhee (9 per cent).

The percentage of damage in selected cultivars of dates ranged from (0 to 45) during the second week with highest damage in the cultivars Mazafati , Hamraya and Deglet Nour (45 per cent) followed by Medjool (42 per cent), Ajwah (39 per cent), Barakawi (30 per cent), Fardh (26 per cent), Jabri (19 per cent) and Barhee (16 per cent).

The percentage of damage in selected cultivars of dates ranged from (0 to 65) during the third week with highest damage in the cultivar Deglet Nour (65 per cent) followed by Hamraya (60 per cent), Medjool (54 per cent), Mazafati (52 per cent), Ajwah (44 per cent), Barakawi (40 per cent), Fardh (34 per cent), Jabri (30 per cent), Barhee (29 per cent) and Khalasah (6 per cent).

The percentage of damage in selected cultivars of dates ranged from (0 to 75) during the fourth week with highest damage in the cultivars Medjool (75 per cent) and Deglet Nour (75 per cent) followed by Hamraya (70 per cent), Mazafati (62 per cent), Barakawi (57 per cent), Ajwah (50 per cent), Barhee (52 per cent), Fardh (52 per cent), Jabri (38 per cent) and Khalasah (11 per cent).

The percentage of damage in selected cultivars of dates ranged from (0 to 85) during the fifth week with highest damage in the cultivars Medjool (85 per cent) and Deglet Nour (85 per cent) followed by Hamraya (82 per cent), Mazafati (72 per cent), Barakawi (63 per cent), Ajwah (63 per cent), Barhee (48 per cent), Jabri (46 per cent), Fardh (45 per cent) and Khalasah (19 per cent).

Among the selected ten cultivars subjected to two months of storage, the mean per cent of damage was found to be higher in the cultivar Deglet Nour (61.00 per cent) followed by Hamraya (57 per cent), Medjool (54.60 per cent), Mazafati (52.40), Ajwah (45.20 per cent), Barakawi (43.40), Fardh (33 per cent), Barhee (29 per cent), Jabri (28.80 per cent) and Khalasah (7.20). The extent of damage increased with increase in duration of storage period. The fruits become highly unpalatable and not suitable for consumption with foul odour and change in flavour.

From the present study it is evident that the extent of damage increased with increase in duration of storage period. The fruits become highly unsuitable for consumption producing foul odour, change in colour and flavour. Fresh dates are perishable and are highly susceptible to losses from damage by stored product insects and causes quantity deterioration between harvest and consumption. The infestation of these exotic fruits leads to loss of quantity, quality and market value was also reported by El-Shafie, 2012.

Biology of major stored product insect pests of dates

The duration of various stages of major stored product insect pests of dates namely *Tribolium castaneum*, *Oryzaephilus surinamensis* and *Carpophilus hemipterus* was presented in Table 3.

Biology of Red flour beetle, *Tribolium castaneum*

Eggs were microscopic, cylindrical and very small. The incubation period ranged from 5 to 11 days. The finding was in accordance with Beeman *et al*,. (2012), who reported the egg period of red flour beetle from 5 to 11 days. The larva was creamy white, translucent with brown head. The duration of the larval period ranged from 19 to 30 days. The pupa was dark brown in colour, dormant and sclerotized. The duration of the pupal period was from 5 to 7 days, which was in accordance with the findings of William (2000). The adults were flattened and reddish brown in colour. The total duration from egg to adult ranged from 33 to 48 days. These current findings stood in line with the reports of (Dhaliwal *et al.*, 2006) and (Rebecca and Thomas, 2003).

Biology of Saw toothed grain beetle, *Oryzaephilus surinamensis*

Eggs were microscopic, oval, elongate, white in colour and deposited in masses. The incubation period ranged from 4 to 5 days, which was previously corroborated by Mason (2010). The larva was yellowish white in colour. The duration of the larval period ranged from 26 to 42 days. The findings goes along with the previous reports by Nelson (1975). Pupa was yellowish white in colour with projections on each side of the thorax. The duration of the pupal period ranged from 9 to 13 days. The adults were narrow, flattened, brown in colour and had six tooth like projections along each side of the thorax. The total duration from egg to adult ranged from 39 to 54 days. Mason (2010) and Nelson (1975) have also corroborated the total life period of saw toothed grain beetle as 39 to 54 days.

Biology of Dried fruit beetle, *Carpophilus hemipterus* : Eggs were microscopic, cylindrical and white in colour. The incubation period ranged from 4 to 8 days. Larva was slender, white with a light brown head. Larval stages were highly active. The duration of the larval period ranged from 18 to 28 days. Pupa was dark brown in colour. The duration of the pupal period ranged from 9 to 14 days. The adult beetles were small with yellow markings on the elytra.

The elytra do not completely cover the abdomen. The total duration from egg to adult ranged from 35 to 45 days. Mason (2010) and Simmons and Nelson (1975) have also corroborated the total life period of red flour beetle as 35 to 45 days.

CONCLUSION

The extent of damage assessed in different cultivars of dates increased with increase in duration of storage period. Maximum damage of 61 per cent was recorded in the cultivar Deglet Nour after two months of storage under laboratory conditions. The infestation of these exotic fruits leads to loss of quantity, quality and market value. The detailed lifestages study of major stored product insect pests of dates revealed the total development period from egg to adult was in the range of 33 to 48 days in *Tribolium castaneum*, 39 to 54 days in *Oryzaephilus surinamensis* and 35 to 45 days in *Carpophilus hemipterus*.

REFERENCES

Abdul Afiq, M.J., Abdul Rahman, R., Che Man, Y.B., AL-Kahtani, H.A. and Mansor, T.S.T. 2013. Date seed and date seed oil. *International Food Research Journal.,* 20(5):2035-2043.

Balinga, M.S., R.Bantwal, V. Kandathil, S. M. Bhat and P. Harshith. 2011. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food Research International.,* 44:1818-1822.

Beeman, R.W., S. Haas and K. Friesen. 2012. Beetle wrangling tips (An introduction to the care and handling of *Tribolium castaneum*. Available online http://www.ars.usda.gov/Research/ docs.htm.

Besbes, S., C. Blecker, C. Deroanne, N.E. Drira and H. Attia, H. 2004a. Date seeds: chemical composition and characteristic profiles of the lipid fraction. *Food Chemistry.,* 84: 577-584.

Besbes, S., C. Blecker, C. Deroanne, G. Lognay, N.E. Drira and H. Attia. 2004b. Quality characteristics and oxidative stability of date seed oil during storage. *Food Science and Technology International.,* 10: 333-338.

Besbes, S., C. Blecker, C.Deroanne, N. Bahloul, G. Lognay, N.E. Drira, and H. Attia. 2004c. Date seed oil: Phenolic, tocopherol and sterol profiles. *Journal of Food Lipids.,* 11: 251-265.

Blumberg, D. 2008.Date palm arthropod pests and their management in Israel. *Phytoparasitica.,* 36(5):411-448.

Buxton, P.A. 1920. Insect pests of dates and the date palm in Mesopotamia and elsewhere. *Bull. Ent. Res.*, 11: 287-303.

Carpenter, J. B., and H.S. Elmer. 1978. Pests and diseases of the date palm (Agricultural Research Service Handbook. No. 527; 42 pp). Washington, DC: United States Department of Agriculture.

Chandrasekaran, M., and H.A. Bahkali. 2013. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology – Review. Saudi *Journal of Biological Sciences.,* 20: 105–120.

Dhaliwal, G.S., Ram, S.,and B.S. Chhillar. 2006. Store grain pest. Issue in Essentials of Agricultural Entomology. pp.356-354.

El-Shafie, H.A.F. 2012. List of arthropod pests and their natural enemies identified worldwide on date palm, *Phoenix dactylifera* L. *Agriculture and Biology Journal of North America.*, 3(12): 516-524.

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley and Sons, New York. 657 p.

Mason, L. J. 2010. Dried fruit beetle (*Carpophilus hemipterus* (L.) and corn sap beetle (*Carpophilus dimidiatus* (L.).*Purdue Extension.*, E-229-W.

Rebecca, B. and R.F. Thomas. 2000. Feature Creatures Entomology and Nematology. http:// entnemdept.ufl.edu/creatures/urban/beetles/r ed_flour_beetle.htm.

Simmons, P., and H.D. Nelson. 1975. Insects on Dried Fruits. Agriculture Handbook 464. Agricultural Research Service, United States Department of Agriculture, Washington, D.C.

William, F.L., 2000. Confused and Red Flour Beetles HYG-2087-97. Ohio State University Extension Fact Sheet Entomology. Available online: http://ohioline.osu.edu/hygfact/2000/2087.htm.

Tables

Table1. Common insect pests of stored dates

S.No.	Scientific name	Common Name	Family	Order
1	*Ephestia cautella* (Walker)	Fig, Almond/ date moth	Pyralidae	Lepidoptera
2	*Tribolium castaneum* (Herbst)	Red flour beetle	Tenebrionidae	Coloeptera
3	*Tribolium confusum* (J.du V.),	Confused flour beetle	Tenebrionidae	Coloeptera
4	*Cryptolestes ferrugineus*(Steph.)	Rusty grain beetle	Cucujidae	Coloeptera
5	*Oryzaephilus surinamensis* (L.)	Saw-toothed grain beetle	Silvanidae	Coloeptera
6	*Oryzaephilus mercator* (Fauv.)	Merchant grain beetle	Silvanidae	Coloeptera
7	*Plodia interpunctella* (Hubn.)	Indian meal moth	Pyralidae	Lepidoptera
8	*Arenipses sabella* (Hmpsn)	Greater date moth	Pyralidae	Lepidoptera
9	*Trogoderma granarium* (Ev)	Khapra beetle	Dermestidae	Coleoptera,
10	*Ephestia figulilella* (Gregson)	Raisin moth	Pyralidae	Coleoptera,
11	*Ephestia elutella* (Hubner)	Tobacco moth	Pyralidae	Coleoptera,
12	*Ephestia kuehniella* (Zeller)	Med. Flour moth	Pyralidae	Coleoptera
13	*Ephestia dowsoniella* (Richard)	Dowson moth	Pyralidae	Coleoptera
14	*Ectomyelois ceratoniae* (Zeller)	The Carob moth	Pyralidae	Coleoptera
15	*Ephestia calidella* (Gunee)	Currant moth	Pyralidae	Coleoptera
16	*Cotinis mutabilis* (Gary& Percher.)	The fig beetle	Scarabaeidae	Coloeptera
17	*Carpophilus hemipterus* (F.)	Driedfruit beetle	Nitidulidae	Coloeptera
18	*Carpophilus decipiens* (Horn)	Dried fruit beetle	Nitidulidae	Coloeptera
19	*Carpophilus dimidiatus* (Fab.)	Corn sap beetle	Nitidulidae	Coloeptera
20	*Carpophilus mutilates,* (Fab.)	Confused sap beetle	Nitidulidae	Coloeptera
21	*Urophorus humeralis* (Fab.)	Pineapple beetle	Nitidulidae	Coloeptera
22	*Haptoncus luteolus* (Erich.)	The yellowish nitidulid	Nitidulidae	Coloeptera

Table 1. Biology of major stored product insect pests of dates

Sl.No	Insect species	Duration in days * (Mean ±SE)			
		Egg Period	Larval Period	Pupal Period	Total life cycle
1.	***Tribolium castaneum***	8.00±0.57	25.80±1.19	6.65±0.24	40.45±1.34
2.	***Oryzaephilus surinamensis***	4.55±0.16	31.60±1.73	10.65±0.39	46.80±2.06
3.	***Carpophilus hemipterus***	6.00±0.37	23.25±0.97	10.90±0.50	40.15±1.06

*Mean of 10 replications

Date palm disorders caused by*Aphomia sabella* Hampson

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Abstract

Several date palm disorders affected date production and cause economic loss to the dates producers. These disorders in date palm are caused either by environmental factors or by biological agents. Certain date palm disorders such as tree crown bending (Barhee disorder), cross cut or 'V' cut, little leaves, white leaf and terminal shoot stunting followed by death were reported as caused by unknown factors. In order to find out the real cause for these disorders, a study was conducted in our laboratory. The main objective of the study was to identify the cause for the above mentioned disorders and find out the remedy. Many affected palms were collected and dissected out in the laboratory or in the field directly for the study. Abnormalities noticed on different parts of each frond while dissection were photographed and recorded. The insects, insect eggs and larvae and insect webbing with brown fecal pellets if any were also collected for identification. Majority of the affected palms showed the presence of same type of insect larvae. This larva was identified as the larva of *Aphomia sabella* Hampson or *Arenepsis sabella* Hampson (greater date moth). Our study confirmed the main cause for the above mentioned date palm disorders was the greater date moth larvae and certain date palm cultivars were more susceptible to these insect larvae. Precautionary measures to protect the palms from these insects and recovery of infected palms were developed. The details of the study are presented herein,

Keywords: *Phoenix dactylifera*, *Arenepsis sabella*, crown bending, Barhee disorder, V cut, cross cut.

INTRODUCTION

Field observations on tissue culture derived date palms indicated certain growth abnormalities or off-types (Zaid and De Wet, 2002) in some tissue cultured plants of few cultivars. The abnormalities found in micropropagated date palms are: changed morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, higher susceptibility to diseases, production of bastard offshoots or Hapaxanthic axillary shoot formation (Sudhersan et al., 2001), hermaphroditism (Sudhersan and Aboel-Nil, 1999) delayed flowering, pollen incompatibility, abnormal fruiting, and parthinocarpy. Among the date palm abnormalities observed, tree crown bending (Barhee disorder), cross cut or 'V' cut, little leaves, white leaf and terminal shoot stunting and sudden dwarfing of date palms at the stage of flowering were occurred in all the three types of palm trees such as: Tissue culture derived palms, offshoot derived palms and seedling derived palms. Since these disorders occurred in all types of field grown date palms, we came to a conclusion that these abnormalities are not related to the tissue culture oriented and motivated the authors to investigate the cause for the above mentioned abnormalities or oftypes.. During our investigation, we have observed insect larvae inside the shoot tip of infected abnormal palms. In order to identify the moth, the larvae were collected and maintained at 28-30•C After a week, the larvae turned to pupae stage and after another two weeks the moth came out. The moth was identified as the greater date moth. The detail about the moth, its mode of action, date palm abnormalities caused by this moth and the economic loss to the farmers are presented herein.

Larva Collection and Identification

Many young date palm trees produced by Tissue Culture (TC) method showed abnormalities or disorders in the field. While dissecting the infected date palms in the field and laboratory, many similar type of insect larvae larvae were observed. These insect larvae ate the tender tissues of young palm trees and affected the growth of the palms. In order to identify the insect larvae, we have collected the larvae in a plastic container and maintained inside the laboratory at 28-30˚C. After a week, the larvae became pupae and the insect came out after another 2 weeks time (Figs.1-4). The insect was identified as the greater date moth *Aphomia sabella* (*Arenipses sabella*).

The Greater Date Moth

Greater date moth is scientifically known as *Aphomia sabella* Hampson (Synonym *Arenipses sabella* Hampson), It belongs to the class Insecta, Order Lepidoptera, Family Pyralidae and the Genus Aphomia. This inset moth is commonly found in North African countries, Iran, Iraq and the Middle East. The host for this moth is the date palm trees (*Phoenix dactylifera* L.). The adult moth is grayish in color and about 2 cm in length. An adult female moth lays about 200-400 eggs at midnight on date palm leaves. The larvae are 2.5 to 4 cm in length and dark gray in color. The larva eats young date palm fronds, primordia, young inflorescences and flower buds. Fully developed larva become pupa stage at 28-30•C and came out as an adult moth (Figs. 1, 2). The larvae are active during March-April and September to October in the middle East countries.

Date Palm Disorders Caused by Greater Date Moth

The greater date moth larvae eat young date palm fronds, tender tissues, young axillary shoot buds and inflorescences causing economic loss to the date palm growers. When the larva eat the tender tissue of the palm shoot tip, the affected palm trees shows abnormalities on the leaves, inflorescences and in the main shoot. The physiological dis orders (abnormalities) such as Sudden dwarfing, Crown bending, frond malformation, malformation of leaflets, V" cut on rachis and inflorescence stalks, and terminal shoot death (Figs. 5-8) of field grown date palms are caused by this moth.

Identification of Infection

The infected palms can be identified through many symptoms such as webbings containing brown colored excreta on the leaves, scratches on the leaf rachis, furrows on the leaf base, "V" cut on the rachis, "V" cut on inflorescence spath and "V" cut on the inflorescence stack. After the severe infection deep inside the tender parts of the shoot tip, symptoms such as abnormal leaflets, wholes on the young fronds, tree crown bending and terminal shoot dwarfing etc. (Figs. 3-6) will appear on the infected trees. If unnoticed, the larvae damage the entire shoot meristem and kill the terminal shoot. We have observed many palm trees suddenly turned into dwarfs died due to this larva infection. After the death of the terminal shoot, the palm produced normal axillary shoots at the basal region where the tree is not affected. Our experimental studies related to this moth larvae confirmed that certain cultivars such as Suckary and Sultana are more susceptible than other cultivars.

Economic Importance

The greater date moth larvae are active during the month of March when the date palm trees start the vegetative and reproductive growth after the winter dormancy. The larvae eat the young fronts and make a long tunnel deep inside the tender part of the tree. Through the tunnel many larvae easily enter inside the leaf axis and grab the tender parts, and create damage to the leaves and inflorescences. The damage to the palm is minor when the number of larvae is less and if the number of larvae are more and they reached the shoot meristem, the damage will be severe. In many palm trees, the inflorescence stalk get damaged by the larva and the whole bunch will be damaged causing economic loss. If unnoticed, they damage the shoot meristem and kill the entire tree. These larvae primarily create wounds on the tender parts of the tree while grabing and afterwards the secondary pathogens (fungus and bacteria) infect through the injured tissue and enhance the damage to the tree growth and production. Nowadays this moth causes severe damage to the date productions in the Middle East.

Control Measures

Monitor the date palm field particularly during the month of February to March for the presence of this moth larvae through the presence of scratches on the fronds and excreted webbings. If it is observed on the trees, spray systemic insecticide inside the shoot tip to save the palms. Generally, the adult moths can be collected through water traps and killed. As a control measure for this moth, chemical control is inevitable solution until finding out an alternative environment friendly bio-control. Many date palms particularly Suckary and Sultana infected by these larvae in our orchard were recovered through applying insecticide and fungicides.

CONCLUSION

The present study confirmed that the above mentioned disorders of date palm such as crown bending, 'V' cut, dwarfing etc are caused by the insect larval attack. The insect was identified as *Arenipses sabella* Hamps. (grater date moth). Generally, the symptoms of these physiological disorders were observed in many unknown date palm cultivars. However, our study confirmed that certain date palm cultivars are highly susceptible to the grater date moth. Among the 20 known date palm cultivars studied in Kuwait, only Suckary and Sultana were affected by this insect larvae. The palms affected by these physiological disorders caused by the larvae could be returned to the normal condition through insect control at appropriate time. As a control measure for this moth, chemical control is inevitable solution until finding out an alternative environmentally friendly method for the control of this greater date moth.

LITERATURE CITED

Sudhersan, C. and AboEl-Nil,M. 1999. Occurrence of hermaphroditism in the male date palm. Palms 43: 48-50.

Sudhersan, C., AboEl-Nil, M. and Hussain,J. 2001. Hapaxanthic axillary shoots in date palm plants grown *in vivo* and *in vitro*. Palms 45: 84-89.

Ziad, A. and P. F. De Wet. 2002. Date palm propagation. In: A. Zaid (eds), Date palm cultivation, FAO Plant Production and Protection Paper No. 156, Rome, Italy.

Figures

Fig. 1. Date moth larva

Fig. 2. Adult moth

Fig.3. "V" cut syndrome

Fig. 4. Shoot bending

Fig. 5. Dwarfing

Fig. 6. Main shoot death

Technical Practices of Date Palm

Date value chain in Saudi Arabia: major obstacles to the international date marketing

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Abstract

The Kingdom of Saudi Arabia is the second world date producing country with a total production of around 1.3 million tons which represents about 14.5% of the total world production. Of the 400 existing date varieties produced in the Kingdom, some figure among the best and most famous in the world and thus, present a high potential for taking the lead in the international date market. Unfortunately, this not yet the case since less than 10% of the total dates exported by Saudi are commercialized on the international market, and most of these exportations are to Arab countries with limited quantities directed to the most profitable markets in Europe and North America. This study aims to present some of the major obstacles facing the international marketing of the Saudi dates and discuss ideas on how to contribute to overcoming these challenges.

Architectural study and simulation of three Algerian date palm (*Phoenix dactylifera* L.) cultivars palms

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Abstract

Architectural parameters were used to describe, simulate and visualize the architecture of palms of three Algerian date palm cultivars 'Deglet Nour', 'Mech-Degla' and 'Ghars'. Biometric and geometric parameters were measured in the field on four different palms per cultivar using the Principes network protocol. The simulation was carried out by the Xplo software developed at UMR-AMAP-CIRAD, simulator of architecture and plant growth, which contains the Palm Principes model. The symmetry study of the left and right sides of palm rib allowed to highlight a slight difference in terms of number and length of pinnae and frequency of their grouping. However, the dissymmetry was more marked by the rotation and axial angles which revealed a localized heterogeneity at 10 and 90% of the palm. Also, the results revealed that the virtual form (3D structure) of the simulated palms was comparable to the actual ones. Indeed, the parameters characterizing the palms of different cultivars appear clearly on the simulated palms namely the lengths of the petiole, the spine and pinnae parts, the number of both leaflets along the rib and spines and also the rotation angles.

Key words: date palm, architecture, symmetry, simulation, characterization, cultivars.

Enhancement the production of agro-biodiversity of date palm (*Phoenix dactylifera* L.) in Siwa Oasis

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Abstract

To generate essential information of the identification, description and documentation the agro-biodiversity of date palm cultivars in Siwa Oasis, taxonomical relationships of fifteen date palm cultivars growing in Siwa Oasis were addressed based on one hundred and three morphological attributes of trunk, crown, leaves, fruits and seeds. The most important attributes are arranged according to their taxonomic significance. Fruit and seed attributes are still the most important criteria to distinguish among date palm cultivars, but the blade, spine and crown attributes are useful in delimitation some cultivars. The main objective of the study is identification of Siwa's date palm varieties according to internationally standardized to combat commercial fraud during offshoots exchange and trade, as well as the ability to apply a certificate of origin for the date palm offshoots / seedlings during exchange and trade. In order to protect farmer's rights and investors in the field of agricultural production of date palm as well as effective sustainable production of date palm, the positive management action must be associated with legislation reform of agribusiness.

Key Words: Biplot - descriptor- morphology- path coefficient.

INTRODUCTION

Date palms (*Phoenix dactylifera* L., Phoeniceae: Caryhoideae) were amongst the first crops domesticated in the Old World (Zohary & Speigel – Roy. (1975). Palms have been cultivated in the Middle East and North Africa for at least 5000 years (Zohary & Hopf, 1988). It was much revered and regarded as a symbol of fertility and of horticultural and economic value in Egypt. It presents a source of income to farmers and creates favorable conditions for improving secondary crop culture like barley, alfalfa and clover as forage (Soliman *et al*., 2003).

Biodiversity conservation of date palm is vital element in sustaining the various number of date palm cultivars in Egypt. A number of neglected and underutilized cultivars of date palm are expected lost forever and consequently loss of date palm diversity in Egypt if it is not regularly maintain (Rizk *et al*.,2009).

Many farmers and investors in the field of agricultural production of date palm might be fall a victim to commercial fraud when they buy seedlings / offshoot and establishment of date palm plantations. So, the identification of date palm varieties according to internationally standardized form (descriptor) is a vital process to sustainable date palm agriculture as well as combat commercial fraud during exchange and trade of offshoots. moreover, the ability to apply a certificate of origin for the date palm offshoots/ seedlings during Exchange and trade. This action will establishment a basis for increasing and sustainable agriculture production of date palm in Siwa Oasis as well as all over Egypt. Indeed, in view of farmers' rights, this action will assist farmers and local communities in Siwa Oasis, as well as areas of high diversity of date palm, in the protection and conservation of their date palm cultivars.

To simplified over view framework on the identification, description and documentation the agro-biodiversity of date palm cultivars in Siwa, systematical relationships of fifteen date palm cultivars growing in Siwa were addressed based on one hundred and three morphological attributes.

It is hoped that the results of the present investigations generate essential information about the status of palms (in situ), its utilization and palms under cultivation (ex situ). We hope it will find application in the preparation of a future account of the "Sustainable Biodiversity of Egypt".

MATERIALS AND METHODS

Fifteen date palm cultivars were checked (*in situ*) and collected for two seasons namely: Arghm Ghazal, Frahi, OlkikWngem, Ghazall, Tazarakht, Karamat, Sewi, Holown Ghanem, Agebeer, Oshek Engebel, Taktakt, Amenzoh, Eghrawn Nehloten & Azwagh.

Following Rizk & El Sharabasy (2007), One hundred and three attributes of trunk, crown, leaves, fruits and seeds selected and measured in this study. These attributes are included those used by Mason (1915), Brown (1924), Brown & Bahgat (1938), Nixon (1950), Al Baker (1962 & 1972), Ibrahim & Hajaj (1993).

The analysis carried out for ten randomly select healthy date palm tree at the same old for each cultivar. Leaf attributes were measured and scored as an average of ten well developed mature leaves. Fruit attributes were scored two times: first at the end of khalal stage and the other at full maturation of soft date palm fruit. Sample of one hundred fruits were picked Randomly from each cultivar for fruit and seed measurements. The terminology of morphological attributes basically follows Stearn (1973).

The statistical analysis and the relationship between the cultivars were measured by calculating their Euclidean distance and complete linkage method as phenogram using SYSTAT version 7.0 (Wilkinson, 1997). The path analysis were measured and calculated accordind to Akintunde (2012).

RESULTS AND DISCUSSIONS

A total of one hundred and Three morphological characters were examined for comparison the date cultivars in Siwa oasis. The fruit and seed characters of the studied date cultivars are shown in plates 1. The most important characters are arranged according to their systematic value as follow:

1) Fruit shape and dimension

Among the studied Siwa date cultivars, there are six fruit shapes can be distinguished as follow:

a) Cylindrical: it’s stout at all length.
b) Elliptical: the two sides are curved equally from the middle, length: breadth ratio 2:1 to 3:2.
c) Ovate elongate: it’s broadest nearly at the middle with length: breadth ratio 2:1.
d) Obovate elongate: it’s broadest nearly at the apex with length: breadth ratio 2:1.
e) Falcoid elongate: the side is curved at the middle.
f) Ovate: it’s broadest below the middle with length:breadth ratio 3:2.

Fruit shape at Khalal stage is an important taxonomic criteria to distinguish between date palm cultivars. It is agreement with Al Baker (1972), Ibrahim & Hajaj (1993) and Amer (2000).

2) Fruit color

The evaluation of fruit color has done in Khalal and mature stage. five colors are determined in Khalal viz.: pale red, Pale yellow, yellow, yellow-brown, orange and five colors are determined in mature viz. yellow-orange mottled pale red, pale brown, brown, brownish-red, brownish black.

Al Baker (1972) mentioned that the fruit color is the most useful criteria in the differential among date palm cultivars.

3) Fruit apex and base

Three types of fruit apex are distinguished in studied date cultivars in Siwa viz.: obtuse, blunt and retuse. While, four types of fruit base are found viz.: obtuse, retuse, truncate and truncate- emarginated.

4) Seed shape

Six shape types can be determined among the studied taxa viz.: cylindrical, elliptical, elliptical-ovate, elliptical-obovate, ovate, and obovate.

Three types of seed apex viz.: obtuse, blunt and retuse.

Seed base can be grouped into five categories viz.: obtuse, blunt, acuminate, caudate, and truncate

5) Micropyle characters

Two characters of seed micropyle provided to be reliable to distinguish between the stated siwa date cultivars.

The most common micropyle position are those towards the apex and in the middle of seed, while the third one, towards the seed base, was observed only in Agebeer. Al Baker (1972) observed the importance of micropyle position in the differentiation among date cultivars.

Regarding micropyle elevation could be categorized into two group:

- superficial
- half sunken

6) Seed / Fruit ratio

Volume and weight of seed / fruit ratio are calculated.

Three categories are stated according to the seed / fruit ratio.

a. Low: less than 0.1
b. Medium: between 0.11 – 0.19
c. High: More than 0.2

The most obvious high volume seed / fruit ratio (0.2) is recorded in Azwagh, while the lowest (0.08) is recorded in Arghm Ghazal.

The high weight seed / fruit ratio (0.290) is recorded in Keabi, while the lower (0.1) is recorded in Agebeer.

There's a positive significant correlation ($r = 0.458905$, $p \leq 0.05$) between weight ratio of seed / fruit– volume ratio of seed / fruit.

7) Perianth

Al–Baker (1972) reported the important of perianth colour and shape in distinguished among date palm cultivars.

The colour of perianth differed from yellow, orange – yellow, and yellow – pale reddish in the studied taxa. Yellow perianth is common among the studied taxa, while Yellow – pale reddish is recorded in Agebeer and karamat.

Perianth apex is divided into three groups: Rounded, Truncate & Emarginate

8) Leaf length

The leaf length ranged from 295 to 645 cm.

The leaves could be divided into 3 groups according to Ahmed *et al.* (1979) as follow:

a) short: less than 325 cm.
b) Medium: from 325 – 425 cm.
c) Long: more than 425 cm.

Sewi cultivar is the only recorded of the short group. In his study on date palm cultivars, Abdalla (1986) mentioned that mature leaves of palms varied according to cultivars.

There's a highly positive significant correlation (r = 0.89079, p≤0.001) between leaf length and blade length.

1A. Fruit Cylindrical. ……..………………..................................... Olkik Wngem
2A. Fruit elliptical …………………….........……………………... Ghazall
3A. Fruit ovate elongate ………….......................……………………………
1B. Seed cylindrical, seed base truncate. ………..............................… Azwagh
2B. Seed elliptical-ovate, seed base acuminate. ……............ Oshek Engebel
3B Seed ellioptical-obovate, seed base blunt. …..………...................... Tazarakht
4B. Seed elliptical, obtuse seed base. …... 1C. Fruit yellow at Khalal, Brown at maturity........................... Agebeer
2C. Fruit orange at khalal, Reddish brown at maturity.. Eghrawn Nehloten
4A. Fruit obovate elongate ………………………………………………......................
1D. Fruit base obtuse, Fruit yellow at Khalal, pale brown at maturity, spine area ratio >30%. ……... Frahi
2D. Fruit base retuse, fruit brownish black at maturity, spine area ratio ≈ 25%.… .………………………...............................…....... Amenzoh
3D. Fruit base truncate. Fruit brown at maturity, spine area ratio ≈ 17% ………………………………………………………………… Keabi
4D. Fruit base truncate emarginated, Fruit pale yellow at khalal, pale brown at maturity, spine area ratio ≈ 20% ….............. Holown Ghanem
5A. Fruit Falcoid - elongate ……………………….………………...................………
1E. Fruit apex and base obtuse, fruit yellow at Khalal, seed elliptical-ovate, Seed apex obtuse, Leaf Long (more than 425cm). .... Arghrm Ghazal
2E. Fruit apex blunt; base trancate, fruit Pale yellow at Khalal, Seed elliptical, Seed apex retuse, leaf short (less than 325 c................ Sewi
6A. Fruit Ovate …………………….……………………………………......................
1F. Fruit base retuse, pale yellow at Khalal changed to pale brown at maturity, seed ovate ……….............………... Taktakt
2F. Fruit base trancate, yellow- orange changed to brownish black at maturity, seed obovate .... Karamat

Data analysis

Cluster analysis was conducted to generate a dendrogram (Figures1 a,b&c) illustrating possible relationships among fifteen date palm cultivars in Siwa based on the most useful morphological attributes.

Regarding fruit attributes (Figure 1a), all taxa are divided into two groups at a distance of 1.111. Within the first group OlkikWngem separates at the distance 0.859. In the second group, Karamat separates from the rest of cultivars at the distance 0.935 followed by Arghm Ghazal and Sewi.

Regarding vegetative attributes (Figure 1b), all taxa are divided into two groups at a distance of 24.789. Within the first group Sewi separates first followed by Holown Ghanem and Olkik Wngem. while Tazarakht and Ghazall are more similar than the other studied cultivars. In the second group, Eghrawn Nehloten separates from the rest of cultivars first, while Frahi and Azwagh is more similar than other studied cultivars.

According to All morphological characters (Figure 1c), all taxa are divided into two groups at a distance of 18.258. Within the first group Sewi was separated at the distance 9.213. Tazarakht is most similar to Ghazall (6.459). In the second group, Eghrawn Nehloten was separated from the rest of cultivars at the distance 15.136. Amenzoh is most similar to Azwagh (0.759) as well as Agebeer is most similar to Karamat (6.861). Frahi and Arghm Ghazal are the most related to each other (1.180).

To get the linkage between the studied date palm cultivars in Siwa and the most important useful morphological attributes, data matrix were standardized and compute coordinates for plotting Biplot mapping by using perceptual mapping (PERMAP) (Figures 2 a&b).

PERMAP-Biplot shows the importance of vegetative attributes splitting the studied date palm cultivars into three groups, the first contains cultivars Arghm Ghazal, Frahi, Oshe; Engebel, Taktakt, Amenzoh, Eghrawn Nehloten, Azwagh and Keabi. the second group contains Olkik Wngem, Ghazall and Tazarakht. the third group contains Karamat, Sewi, Holown Ghanem and Agebeer which has a common attributes between group one and three.

PERMAP-Biplot shows the importance of Crown shape (3), Leaflet density (25), Spine area length (41), Spine area/leaf ratio (42), Spine gradually (52) and Spine apex (54) to group Frahi and Azwagh. while regarding fruit attributs, Fruit denisty (5), Skin thickness (18), Ventral furrow nature (43), Flesh texture (21) and Weight of seed / weight of fruit (33) are the most important to differentiate between them.

Leaf curvature (8), Blade/leaf ratio (23), Fruit color (khalal) (9), Seed apex (35) and Micropyle position (39) are most important to distinguished Sewi from the rest of studied cultivars. the vegetative data show that the cultivar Agebeer has a common attributes to soft studied cultivars, while fruit data showing the nearest to dry date palm cultivars.

The path coefficient method was being applied in all works of life. Path Analysis extends multiple regression analysis, but while regression gives the best or closest prediction of the response variable based on the given causal factors by the method of least squares, path analysis goes further by providing probable interpretation of the relationships between and within the contributing causal factors to the observed effects. The Direct and indirect path coefficient of some fruit characters on fruit volume were stated in table (1). The seed volume has a positive direct effect on fruit volume reaches 1.1719, while indirect effect reaches 5.9380. The most indirect effect of seed weight via seed volume on fruit volume reached (1.0407), followed by seed width (0.9245) and fruit width (0.9094) and flesh thickness (0.8518).

CONCLUSION

In fact, Traditional date palm cultivars affected and consequently threatened by horticultural systems, ethics of trade and socioeconomic factors of local community. It is necessary to emphasize that date palm cultivars, in fact, demand urgent management action to conserve its threatened and unique biodiversity. Positive conservation action may be necessary at the infra-specific level if diversity of date palm cultivars are to be maintained. The active cultivation is vital to survival date palm diversity and a cultivars are soon lost forever if it is not regularly propagated. The first and important step to conserve date palm biodiversity is standard identification of date palm cultivars and its value. Because of the human cultural of local communities have a heritage associated with date palm, the conservation and sustainable utilization of date palm diversity must be considered as a societal enthusiastic. In order to protect farmer's rights and investors in the field of agricultural production of date palm as well as for effective sustainable production of date palm, the positive management action must be associated with legislation reform. Due to control the date palm offshoots / seedlings trade to combat commercial fraud during exchange and trade of offshoots. moreover, the ability to apply a certificate of origin for the date palm offshoots/ seedlings during Exchange and trade.

In view of socio-economic impact as ensuring the identification of date palm cultivars, it will increase the Profitability Index (economic return) from date palm productions and strengthen the inter-stakeholders collaboration in Siwa oasis as well as all over Egypt including government, civil society, private sector, farmer and local communities concerning conservation and sustainable use as well as increasing production of date palm genetic resources. Finally, sensitize farmers as well as local communities about the importance of apply a certificate of origin and using legal identified date palm cultivars.

REFERENCES

Abdalla, M.Y. 1986. Morphological and chemical studies through flowering and fruiting stages of date palm. Ph.D. thesis, Faculty of Agriculture, Cairo university.

Ahmed, F.H.; El Qahtani, M. & Wali, Y.A. 1979. Cultivation and production of Tamr date palm in Arabic and Islamic countries. Ain shams university press, Cairo, Egypt.

AKINTUNDE, A. 2012. Path Analysis Step by Step Using Excel, Journal of Technical Science and Technologies, 1(1):9-15.

Al Baker, A.J. 1962. Iraq Tamr palm Iraq. Government press, Baghdad, Iraq. (In Arabic)

Al Baker, A.J. 1972. Date Palm, Past and Present, The New Cultivation, Manufacturing And Trade. Al–Ani press, Baghdad, Iraq (In Arabic)

Amer, W.M 2000. Date palm, *Phoenix dactylifera* L. Cultivars in Egypt . *El Minia science Bulletin*, volume 13(1) 1-15.

Brown, T.w. & Bahgat, M. 1938. Date palm in Egypt . Booklet 24, Ministry of Agriculture, Horticultural section, Cairo.

Brown, T.w. 1924. Date palm in Egypt. Technical of Agriculture, Horticultural section 43: 11-15

Ibrahim, A.M. & Hajaj, M.n. 1993 . Tamr palm, cultivation and production . pp. 650. Maaref, Alexandria, Egypt (In Arabic)

Mason, S.C. 1915. Botaincal characters of the leaves of the palm used in distinguishing cultivated varieties. *USDA. Bull*. 223 : 1- 28.

Nixon, R.W. 1945. Date cultvre in the united states . *USDA Circ*. 728.

Rizk, R.M, and EL-Sharabasy, S.F. (2007). A Descriptor for Date Palm (Phoenix dactylifera l.) Characterization and Evaluation in Gene Banks. Plant Genetic Resources Newsletter of Bioversity International, 150:52-44, 2007

Rizk, R.M.; El Sharabasy, Sh.; A.M. Abou-Talb and A.A. El-Bana. (2009). The impacts of Economic value on the sustainability of date palm (*Phoenix dactylifera* L.) cultivars in Egypt. Egyptian Journal of Agricultural Economics, 19 (2) 624-639.

Soliman, S.S., Ali, B.A., and Ahmed M.M.M. 2003. genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. African J.Biotech. **2**:86-87.

Stearn, W.T. (1973). Botanical Latin. Newton Abbott: David & Charles.

Wilkinson, L. 1997. SYSTAT*:* The System Analysis For Statistics. SYSTAT, Evanston, III

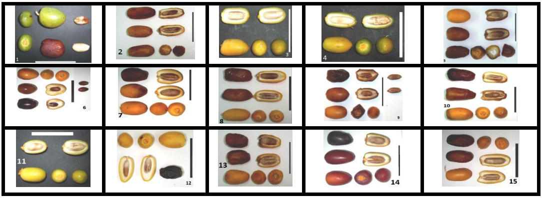
Zohary, D. & Hopf, M. 1988. Domestication of plants in the old world: the origin and spread of cultivated plants in west Asia, Europe and the Nile valley . Clarendon press, Oxford.

Zohary, D. & Speigel - Roy, P. 1975. Beginning of fruit growing in the old world . *science* 187: 319 – 327.

Tables

Table 1 : Direct and indirect path coefficient of some fruit characters on fruit volume.

	Direct path coefficient	indirect effect	Fruit Length	Fruit Width	Fruit Weight	Flesh thickness	Seed Length	Seed Width	Seed Weight	Seed Volume	Weight of seed/ weight of fruit	Volume of seed/ volume of
Fruit Length	0.0404	0.9124	**0.0404**	0.0163	**0.0233**	**0.0181**	**0.8353**	**0.0338**	**0.0057**	0.0110	**-0.0180**	**-0.0131**
Fruit Width	0.1316	0.4583	0.0531	0.1316	0.1127	0.0455	0.0149	0.0284	0.0729	0.1021	**-0.0531**	**0.0034**
Fruit Weight	0.3597	1.8962	0.2075	0.3079	0.3597	0.8142	0.3869	0.0908	0.1289	0.2082	**-0.2297**	**-0.0704**
Flesh thickness	-0.3060	-1.1464	-0.1372	-0.2618	-0.2491	-0.3060	-0.0402	-0.1237	-0.1614	-0.2224	**0.1192**	**-0.0130**
Seed Length	-0.0408	-0.1206	-0.0341	-0.0115	-0.0158	-0.0120	-0.0408	0.0003	-0.0144	-0.0143	**0.0011**	**0.0089**
Seed Width	0.0707	0.3198	-0.0106	0.0378	0.0179	0.0286	-0.0005	0.0707	0.0636	0.0558	**0.0310**	**0.0251**
Seed Weight	-0.4109	-1.9016	-0.0575	-0.2277	-0.1473	-0.2168	-0.1449	-0.3693	-0.4109	-0.3649	**-0.1832**	**-0.1483**
Seed Volume	1.1719	5.9380	0.3198	0.9094	0.6785	0.8518	0.4094	0.9245	1.0407	1.1719	**0.1717**	**0.5009**
Weight of seed/ weight of fruit	0.2247	0.0984	-0.1002	-0.0907	-0.1435	-0.0875	-0.0063	0.0986	0.1002	0.0329	0.2247	**0.1031**
Volume of seed/ volume of fruit	-0.6673	-0.9829	0.2168	-0.0171	0.1306	-0.0284	0.1453	-0.2367	-0.2409	-0.2852	-0.3062	**-0.6673**



Figures

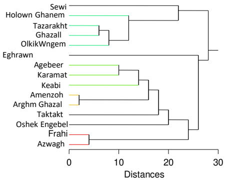


Figure 1b : Phenogram showing the relationships among 15 date palm taxa based on vegetative attributes, using Euclidean distance and Median linkage

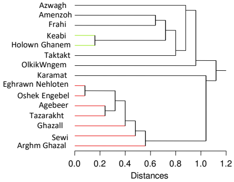
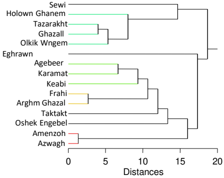


Figure1a: Phenogram showing the relationships among 15 date palm taxa based on morphological attributes (fruit), using Euclidean distance and Median linkage method.



Figgure1c : Phenogram showing the relationships among 15 date palm taxa based on all morphological attributes (vegetative and fruit), using Euclidean distance and Median linkage method.

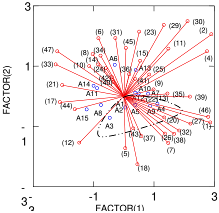


Figure 2a: Perceptual mapping (Biplot) of the studied Siwa date palm cultivars for combination of taxa and fruit attributes, Configuration has been standardized. For cultivars name see pate 1.

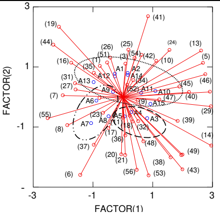


Figure 2b: Perceptual mapping (Biplot) of the studied Siwa date palm cultivars for combination of taxa and vegetative attributes, Configuration has been standardized. For cultivars name see plate 1.

Predicting farmers' willingness to adopt liquid pollination and polycarbonate drying house technologies: a case study from the date palm growers in the Sultanate of Oman

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Abstract

The aim of this research paper is to assess the adoption level of the two technologies (liquid pollination and polycarbonate drying houses) in the Sultanate of Oman with emphasis on identifying influencing factors of the adoption process and exploring resulting policy implications. The methodological framework used is based on the implementation of the ADOPT (Adoption and Diffusion Outcome Prediction Tool) tool in two localities of the Sultanate of Oman through focus groups discussion (FGD's).

Empirical findings obtained from the assessment of the LP technology indicate that peak adoption rate for liquid pollination technology in "North Al Batinah" is high and predicted to be around 95% (of the total population) after a period of 14.5 years. The predicted adoption level after 5 and 10 years from introducing the technology in the region is expected to be 46.9% and 91.5%, respectively. The assessment of the rate of adoption of the Polycarbonate Drying Houses (PHD) technology and the identification of factors affecting the peak and adoption levels, and constraints that limit the adoption process and widespread of such technology among the date palm growers of Oman indicates that peak adoption rate for PDH technology in the target study region is predicted to be 95% after a period of 21 years. The predicted adoption level after 5 and 10 years is expected to be 23.5% and 72.9%, respectively.

The presented results suggest that sustainable increase in date palm productivity can be achieved if farmers are encouraged to adopt the LP and PDH technologies. However, the adoption of such technology needs to be accompanied by a supporting extension system and an enabling policy environment to ensure the scaling-up and widespread use of these promising and profitable technologies.

Key words: Adoption, liquid pollination, polycarbonate drying houses, date palms, FGD's, ADOPT, Oman.

1. INTRODUCTION AND BACKGROUND

Within the framework of the project "*Development of sustainable date palm production systems in the GCC countries of the Arabian Peninsula*", funded by the GCC Secretariat, researchers succeeded to introduce two promising technologies: liquid pollination (LP) and polycarbonate drying houses (PDH). The aim to introduce LP technology is to improve the quality of fruits, reduce and save the time and effort during the pollination operation, reduce the risk of low fruit setting by pollination during the peak period of flowering, contribute to reducing harvesting losses. Therefore, the objective to introduce PDH technology is to improve the quality of dried dates, accelerate their drying process, and obtain cleaner fruits that are free from dust. The justification for solar driers is that they are more effective than sun drying traditional system (*Mistah*), with lower operating costs than mechanized drier.

These technologies have received a great deal of attention from the Government decision makers in recent years, but there is still no clear assessment of its current level and intensity of adoption, and the factors affecting its adoption. The success of both technologies will not only depend on how well from a technical perspective, but also on its affordability and profitability. The utilization and critical mass adoption of appropriate innovations is an important prerequisite for agricultural development, particularly in the Cooperation Council for the Arab States of the Gulf (GCC) countries in general and in the Sultanate of Oman, in particular.

The aims of this research paper is to assess the adoption level of the two technologies in the Sultanate of Oman with emphasis on identifying influencing factors of the adoption process and exploring resulting policy implications.

2. Date palm sector in the Sultanate of Oman: Setting the scene

Date palm (*Phoenix dactylifera* L.) is a major fruit crop in the Arabian Peninsula, where it has been closely associated with the life of the people since pre-historic times. Date palm is a multipurpose tree used for food, feed, and fuel (fire wood). It provides fiber, carbohydrates, minerals, and vitamins besides having certain medicinal properties (Al-Farsi et al., 2005; Al-Yahyai and Khan, 2015). In Oman, date palm is the primary agricultural crop, and it constitutes 80% of all fruit crops produced and 50 % of the total agricultural area in the country (FAO, 2013). Oman is the eighth largest producer of dates in the world with an average annual production of 260,000 tons per annum. There are approximately more than over seven million date palms and 250 cultivars in cultivation. However, around 70 % of the total date production is harvested from only 10 cultivars, and a small fraction (2.6%) of the total date production is exported. Only half of the dates produced are used for human consumption, with the other half being utilized primarily for animal feed or considered surplus and wasted (Al-Yahyai and Khan, 2015).

According to Al-Marshudi (2002) and Al-Yahyai (2007), the yield of the date palm is considered to be low (40-80 kg/tree) compared to the yields in neighboring countries (i.e. Saudi Arabia and UAE). This low yield is a result of traditional management, lack of farmer know-how, high infestation by several pests, limited field expansion because date growing regions are fully dependent on groundwater extraction for irrigation, in addition to logistic problems, including an insufficient number of skilled laborers and underdeveloped facilities (transport, storage, market outlets, and large processing factories).

3. Liquid Pollination Technology (LPT) in the Sultanate of Oman: An Appraisal

3.1. Characteristics of the LPT

Pollination of date palm is normally carried out by hand in almost all date palm groves in Oman. Farmers are unaware of Liquid pollinationwhich may be easiest and most productive and convenient. According to Al-Yahyai and Khan (2015), there are several male palm cultivars that are used for pollination, most notably *Khoori* and *Bahlani*. El Mardi *et al.* (2002) pollinated varieties of date palm by hand, and using a hand duster and motorized duster with no effect on fruit yield, despite the larger fruit volumes when dusters were used. They also reported that a pollen/flour (1:5) ratio for mechanical pollination produced lower sucrose and dry matter and a higher yield. In this regard, the project develops a new liquid pollination technology.

3.2. Advantages of using LPT

The advantages of using LP technology in the Sultanate of Oman are
as follows:

- Saves time and effort (reducing labor cost and improving the effectiveness and productivity of the labor used);
- Reduces the quantity of pollen needed;
- Reduces labor and pollen costs;
- Reduces the risk low fruit setting by pollinating during the peak period of flowering;
- Improves the quality of the fruits and consequently the profitability of the varieties intended for export;
- Contributes to reducing harvesting losses;
- Reduces the risk of climbing accidents to laborers.

3.3. Constraints of Using LPT

With respect to the main constraints of using the LP could be
as follows:

- No interest from the younger generation in date palm production;
- The pollination extraction device is expensive (around OMR3500), which small-scale farmers cannot afford;
- Limited number of date palm trees per farmer (the investment in the pollination extraction device is not profitable);
- Resistance of farmers to adopting the new technology and to changing their practices (farmers are accustomed to the old technology of hand pollination);
- Lack of specialized extension services for the date palm;
- Limited number of extension staff with massive responsibilities.

3.4. Socio Economic Evaluation of LPT

The intervention introduced by the project for the pollination of date palm trees was evaluated economically against the manual method for the *Fardh* cultivar based on the data collected from researchers and experts at the Date Palm Research Center, Experimental and Research Farm – Wadi Quriyat. In the findings reported in Dhehibi *et al.* (2016a), it was assumed that the yield will be maintained the same using the two options (LP technology and manual pollination). The premise that even if the quantity produced of dates is slightly reduced using liquid pollination, the weight of fruit will increase – given the advantage of a decreased proportion of the fruit setting and concomitant increase in the quality of the fruit. In this case it was considered as natural fruit thinning. This improvement in the quality will affect the market price and for that it was considered a higher price for the dates produced using liquid pollination. From this research study, it was found that a reduction in pollination cost using liquid pollination was observed in comparison to that for manual pollination of about 89.05% and, consequently, a reduction in the total variable costs per hectare against those for manual pollination of about 56.48%.

Moreover, the analysis revealed a total reduction in the variable costs of OMR1273.95 from using liquid pollination. This reduction in total variable costs results from an increase in the net revenue over that resulting from manual pollination of OMR2593.95/ha. Economic indicators showed also the clear profitability of using liquid pollination where the percentage change in net returns is very high (+ 674.71%). The benefit-cost ratio (BCR) is three times higher when using liquid pollination. Thus, with an internal rate of return of 12.04 and higher BCR, it was concluded that liquid pollination will be highly profitable for Omani farmers.

From the same study, it was reported also that similar results were achieved from the data obtained from farmers for the *Khalas* cultivar. With the same assumptions on yield and related price-quality, it was found that an increase in the value of production of about 20% from using liquid pollination rather than the manual pollination. The analysis showed that using liquid pollination reduced the pollination operation costs by 89.05% (which is the equivalent of OMR1273.95/ha) compared to traditional pollination. The reduction in pollination induces a reduction in the total variable costs of 22.10%. Economic analysis results revealed also that the net benefit to date palm farmers, using the cultivar *Khalas*, and applying liquid pollination was OMR15,310.5/ha (an increase of around 42.60% compared to manual pollination). The analysis of the Internal Rate of Return (IRR) indicates that investment in liquid pollination technology is a profitable decision. Generally, using LP will yield a cost-benefit ratio that reaches 3.41, which is almost twice the ratio obtained from using manual pollination.

4. Polycarbonate Drying House for Date Palm Products (PDH) Technology in the Sultanate of Oman: An Assessment

4.1. Characteristics of the PDH for Date Palm Products

The PDH dryer is a unique cost efficient method of drying agricultural products such as date palm products at commercial scale. It consists of a drying chamber and an exhaust fan. The roof and the wall of a PDH are made by transparent plastic films that are mounted on a metal frame.

Shahi *et al.* (2011) found that the solar drier sheet has a transmissivity of approximately 92% for visible radiation which traps the solar energy during the day and maintains an optimum temperature for drying of produce. In addition, the authors indicated that UV-stabilized films play an important role in PDH dryers. The UV radiation in the sun rays tends to cause changes in the organoleptic properties such as texture, color and flavor of food materials (Shahi *et al.,* 2011). From technical characteristics, UV-stabilized polyethylene sheets are used to prevent such deterioration, and consequently the sheet allows only short wavelength which is converted into long wavelength when it raids on the surface of the dried product. Since the long wavelength cannot move out, it increases the temperature inside the dryer. In addition to the outlined advantages mentioned above, the sheet has superior properties in terms of transparency, transmissivity, property, anti-corrosion, tensile properties, tear-resistant, anti-puncture, water proof, moisture proof, and dust-proof.

According to Janjai *et al.* (2011), polycarbonate covers have been used recently for PDH construction. Contrary to the polycarbonate, plastic sheets and glass covers have the distinct property to allow light to enter the PDH dryer and retaining it inside the chamber, the heating mechanism is as black surface inside the PDH improves the effectiveness of converting light into heat. Hence, the objective of a PDH dryer is to maximize the utilization of solar radiation. Based on the mode of heat transfer, the technology is classified into passive and active PDH dryers. The passive mode dryer works on the principle of thermosyphic effect i.e. the moist air gets ventilated through the outlet provided at the roof of the dryer (Janjai *et al.,* 2011).

Sangamithra *et al.* (2014) showed that trapped light is converted into heat energy to remove moisture from dates in the PDH dryer. The dryer can be connected in series and hence its capacity can be enhanced as per requirement and it can be dismantled so that its transportation is easy from one place to another. Prakash and Kumar (2014) indicated that for active PDH dryer, there are two energy sources namely the air saturation deficit and the incident global solar radiation. Both natural and forced convection methods circulate the hot air to the food material. One of the differences is that, at the initial stage of drying, the value of mass transfer coefficient is double in the active mode than in passive PDH dryers.

4.2. Advantages of using the PDH for Date Palm Products

The principal advantages on using the PDH technology are the following:

- Improves the quality of the fruits, especially in humid areas;
- Avoids the contamination of dates by insects, birds, dust, and rain;
- Accelerates the drying process;
- Reduces the loss rate;
- Could be used for other purposes (e.g. drying other products, such as fish).

4.3. Constraints to using the PDH for Date Palm Products

Although the high range of advantages on using the PDH technology, some constraints or limitations still exist and could be as follows:

- High initial investment cost (needs to be subsidized by the government);
- Concerns over the impact of heat on the quality of product (transfer of the plastic material);
- Farmers lack knowledge on the maintenance of the system;
- Not profitable for date palm growers with very small holdings;
- Lack of extension agents specialized in date palm.

4.4. Socio Economic Viability of PDH for Date Palm Products

The traditional methods used in Oman for drying dates under direct sunshine called "*Mustah*" is a slow process with problems like dust contamination, insect infection, bad quality of fruits, and spoilage due to unexpected climatic changes. To overcome this problem, one of the main objectives of the "*Development of Sustainable Date Palm Production Systems in GCC*" project is to produce new knowledge and practices to improve date palm production systems in the Gulf region.

Other alternative options are available to overwhelm the problem such as the use of conventional fuel fired or electrically operated dryers. However, in many rural areas, the supply of electricity is not available or it is too expensive and could not be affordable by the small date palm growers for drying purpose. Moreover, the fossil fuel fired dryer's technology possesses several financial barriers due to large initial investment and operational running cost which are beyond the reach of small farmers. The main objective if introducing this technology by this development project was to improve the quality of dried dates, accelerate their drying process, and obtain cleaner fruits that are free from dust.

This technology is considered to be one of the most attractive and promising applications of solar energy systems in the GCC countries can be utilized in date palm production areas as a better alternative to dehydrate the date and other agricultural products without any difficulties. Also and from and environmental perspective, the use of PDH can result in reduced emissions if conventional fuel is replaced.

The implementation of this improved technology can have positive socioeconomic impacts on local income generation, food security and consequently a sustainable date palm farming system. In the practice, Chavada (2009) found that the lifetime cost of drying with solar power is only a third of the cost of using a dryer based on conventional fuels. According to Janjai *et al.* (2009, 2011), the price of dates dried in PDH was found to be 20% higher than that obtained from the open sun drying. The estimated payback period (PBP) of the former technology was 2.3 years. Dhehibi *et al.* (2016) found that a PDH dryer can function successfully and efficiently with minimum maintenance at low cost.

With no further disadvantages, it could be a substitute to the conventional dryers thereby making it assessable and affordable by local farmers in the Omani date palm producers. In this study, PDH dryer for dates were evaluated economically for two types (small vs large PDH) under two scenarios: with and without governmental subsidies. Empirical findings reveal the high profitability of the PDH, even when it is not subsidized by the government. At a real discount rate of 5.1%, the net present value (NPV) is positive and very high in all cases. Thus, such an investment is usually acceptable if the NPV is positive (the investment is profitable). This criterion was also supported by both the IRR and the PBP criteria (Figures1-4).The estimated IRR was higher than the current interest rate in the Sultanate, which could encourage both date palm growers and private investors to invest in polycarbonate drying houses. The PBP was found, in the worst case scenario, to be 3.77 years, which is relatively short considering the life of the system (15-20 years). This suggests that investment or action costs in this dryer system are recovered quickly reducing the risk involved in the investment.

5. Adoption Assessment of LP and PDH Technologies in the Sultanate of Oman

5.1. Conceptual Framework

The adoption of new agricultural technologies has generally been found to be a function of farm and farmer characteristics and specific features of the particular technology (Feder et *al*., 1985; Marra and Carlson, 1987; Rahm and Huffman, 1984). A considerable set of literature was developed regarding factors thatinfluence the adoption of new technologies by farmers through use of innovation theory (Feder et *al*., 1985; Griliches, 1957, and Rogers, 1995). Adoption and diffusion theory also have been widely used to identify factors that influence an individual's decision to adopt or reject an innovation. Rogers (1995) defined an innovation as "…an idea, practice or object that is perceived as new by an individual or other unit of adoption. The perceived newness of the idea for the individual determines his or her reaction to it". He further identified five characteristics of an innovation that affect an individual's adoption decision: (i) Relative advantage: how the innovation is better than existing technology; (ii) Compatibility: the degree to which an innovation is seen as consistent with existing experiences, needs, and beliefs of adopters; (iii) Complexity: how difficult the innovation is to understand and use; (iv) Trialability: the degree to which the innovation may be used on a limited basis; and (v) Observability: the degree to which the results of an innovation are visible to others.

The relative advantage and observability of an innovation represents the immediate and long-term economic benefits from using it, whereas compatibility, complexity, and trialability indicate the ease with which a potential adopter can learn about and use an innovation (Boz and Akbay, 2005; King and Rollins, 1995). As the relative advantage, compatibility, complexity, trialability, and observability of liquid pollination and polycarbonate drying house have caused more farmers to adopt them in the GCC countries, in general and, in the Sultanate of Oman, in particular, we can consider the adoption of the two technologies as an innovation. The utilization and critical mass adoption of such technologies is an important prerequisite for agricultural development, particularly for the date palm producing countries in the Arabian Peninsula.

5.2. Methodological Framework: Adoption Analytical Model: Adoption and Diffusion

Outcome Prediction Tool (ADOPT) [1]

ADOPT is an MS Excel-based tool that evaluates and predicts the likely level of adoption and diffusion of specific agricultural innovations for particular target population. The tool uses expertise from multiple disciplines to make the knowledge about adoption of innovations more available, understandable and applicable to researchers, extension agents and research managers. ADOPT predicts the proportion of a target population that might adopt an innovation over time (Figure 5).

The tool makes the issues around the adoption of innovations easy to understand. ADOPT is useful for agricultural research organizations and people interested in understanding how innovations are taken up. The tool has been designed to:

1. **Predict** the likely peak level of adoption of an innovation and the time taken to reach that peak.
2. **Encourage** users to consider the factors that affect adoption at the time that projects are designed.
3. **Engage** research, development and extension managers and practitioners by making adoptability knowledge and considerations more transparent and understandable.

ADOPT users respond to qualitative and quantitative questions for each of twenty-two variables influencing adoption. Going through this process also leads to increased knowledge about how the variables relate to each other, and how they influence adoption and diffusion. ADOPT is structured around four categories of influences on adoption (Figure 5 above): (1) Characteristics of the innovation; (2) Characteristics of the target population; (3) Relative advantage of using the innovation; and (4) Learning of the relative advantage of the innovation.

[1] All information concerning how ADOPT works is found at: http://aciar.gov.au/files/node/13992/adopt_a_tool_for_evaluating_adoptability_of_agric_94588.pdf.

5.3. Data Collection and Data Sources

The study took place in two governorates in the Sultanate of Oman (South and North Al Batinah) characterized by an extensive date palm production and the common testing of the liquid pollination technology and implementation of the polycarbonate drying houses. The data were collected using focus group discussion (FGD) methodology (Krueger, 2002) to apply the ADOPT tool (Kuehne et al., 2013) with a group of farmers in the two Governorates. To assess the liquid pollination technology, we interviewed 24 date palm growers divided in two equal FGD's, each covering 12 farmers'. For the polycarbonate drying house technology, a group composed of ten (10) farmers was also interviewed. The study took place in the two governorates during January 2017.

We also organized a FGD with Ministry technical staffs representing both Agricultural Development Centers. All of them were males. One researcher from the Omani Date Palm Research Centre, the date palm project manager and the socio economic leader of the project economic activities from the International Center for Agricultural Research in the Dry Areas (ICARDA: http://www.icarda.org) conducted the FGD with farmers. In the two cases, we streamlined 22 discussion questions around four categories of influences on adoption. The format of the discussion group consisted of both analytical questions (i.e., they discuss and collectively decide what they believe the answer is), and clarifying questions (i.e., questions that help clearing up confusion and explain why they had chosen this answer). Farmers were asked to think about their problems related to implementing liquid pollination and the most challenging for them.

6. RESULTS AND DISCUSSION

6.1. Factors Influencing Adoption Level and Time to Peak Adoption Level of LP

Technology The issue of this technology adoption by agricultural producers has not been assed. This study has generally focused on the technology adoption processes at the firm level and on identifying the main factors affecting its adoption process. The results of the program predicted that 95% of the South and North Al Batinah Communities would adopt the innovations after 16.9 and 14.5 years, respectively (Table 1).

As displayed in the table above, the peak adoption rate for liquid pollination technology in the "North Al Batinah" is predicted to be 95% after a period of 14.5 years. The predicted adoption level in 5 years and 10 years from start is expected to be 46.9% and 91.5%, respectively. In "South Al Batinah" Governorate, the predicted adoption levels are similar. Indeed, the predicted years to peak adoption is 16.9 years and the peak level of adoption is around 95%. This peak is predicted to be 35.8% and 85.8% after 5 and 10 years from start, respectively.

Results from the sensitivity analysis (Figures 6 & 7) indicates that farmers' conditions of severe short-term financial constraints, the triability of the innovation on a limited basis before a decision is made to adopt it on a larger scale, the perception and evaluation of the liquid pollination technique; i.e. how the innovation allow the effects of its use to be easily evaluated when it is used, the paid advisory delivery system, the development of substantial new skills and knowledge to use the innovation by the farmers, and finally the size of the up-front cost of the investment relative to the potential annual benefit from using the innovation are the driving adoption factors for the liquid pollination technology in the two targeted areas.

6.2. Factors Influencing Adoption Level and Time to Peak Adoption Level of PDH Technology

The predicted years to peak adoption and the predicted adoption level, including the level in 5 and 10 years from start, is presented in Table 2. Even though adoption and diffusion of the PDH dryer is very difficult to forecast— the issue is complex and crosses economic, social and psychological disciplines—there is an ongoing need and demand for specific estimates to be made.

Empirical findings from the table below revealed that 95% of "South Al Batinah" Community would adopt the innovations after 20.9 years. However, the predicted adoption levels after 5 and 10 years from start is 23.5% and 72.9%, respectively. Even though the time to peak adoption was longer than what we expected (bearing in mind that this figure affected the attractiveness of the technology in the future funding), these results are expected since the upfront cost of investment is quite high while the economic viability of this technology make the evidence of its profitability. Indeed, the outcomes from this tool could be considered as real values to inform the different stakeholders about the influences on adoption and diffusion of the PDH technology in Oman.

After presenting these indicators, the FGD's outputs discussion outlined that farmer's most commonly cited motivations for adopting this technology although the high upfront cost of investment. Our study and FDG's discussion found that both adopters and non-adopters saw the greatest benefits of this technology in terms of its potential benefit on the quality of the final agricultural dried products (dates, in this case). Another way to better understand the factors associated the rapid and large adoption of the PDH technology was by conducting a sensitivity analysis. Important factors to farmer decision making differ according to geographic, economic, and social context.

However, taken together, the results from the sensitivity analysis regarding the main factors affecting the adoption decision of PDH technology in AL Batinah Governorate are displayed in Figure 8. The figure content indicates that triability of the innovation on a limited basis before a decision is made to adopt it on a larger scale, the perception and evaluation of the PDH technique; i.e. how the innovation allow the effects of its use to be easily evaluated when it is used, the paid advisory delivery system capable of providing advice relevant to the use and management of the technology, and finally the size of the up-front cost of the investment relative to the potential annual benefit from using the innovation are the driving adoption factors for the PDH technology in the target area.

7. Concluding Remarks and Policy Implications

The objective of this paper is to analyze the main factors affecting the predicted adoption level, the peak to reach this level, and the constraints of adoption of LP and PDH technologies introduced by the date palm project in the sultanate of Oman. The methodological framework used was based on the implementation of the ADOPT tool to focus groups of date palm growers in two localities of the Sultanate. In the FGD we streamlined 22 discussion questions around four categories of influences on adoption: characteristics of the innovation, characteristics of the target population, relative advantage of using the innovation and learning of the relative advantage of the innovation.

The empirical findings obtained from the liquid pollination technology assessment indicates that peak adoption rate for liquid pollination technology in "North Al Batinah" is predicted to be 95% after a period of 14.5 years. The predicted adoption level in 5 years and 10 years from start is expected to be 46.9% and 91.5%, respectively. In "South Al Batinah" Governorate, the predicted adoption levels are similar. Indeed, the predicted years to peak adoption is 16.9 years and the peak level of adoption is around 95%. This peak is predicted to be 35.8% and 85.8% in 5 and 10 years from start, respectively. The assessment of the rate of adoption of the PDH technology and the identification of factors affecting the peak and adoption levels, and constraints that limit the adoption process and widespread of such technology among the date palm growers of Oman indicates that peak adoption rate for PDH technology in the target study region is predicted to be 95% after a period of 21 years. The predicted adoption level after 5 and 10 years is expected to be 23.5% and 72.9%, respectively.

The presented results suggested that sustainable increases in productivity of date palm in the Sultanate of Oman can be achieved if farmers are encouraged to adopt the liquid pollination and polycarbonate drying chambers technologies. However, the adoption of such technology needs to be accompanied by a supporting extension system and an enabling policy environment to ensure the scaling-up and widespread use of this promising and profitable technology. Such findings can provide a useful framework for decision-making as date palm producers and policy makers confront sustainable date palm farming system. In addition, the results can facilitate the policy formulation process as policy makers, responding to societal pressures, attempt to move date palm farming system in a more sustainable direction while trying to improve the profitability of the sector, in general. Implications can be derived for producers for whom local environmental quality is closely linked to date palm production systems in Oman. The results from the present research study suggest the following:

- Creation of private service companies to carry out and monitor the LP operations. These companies can even be operated by small farmers in order to diversify their income sources;
- Enhancing the extension services (more and specialized extension agents) and the development of an effective extension service for Omani date palm growers;
- Reinstatement of the subsidy system in the sector;
- Creation of private services and marketing companies with support from the government;
- Enhancing the awareness of farmers regarding the profitability of using this technology in comparison to the manual pollination method;
- Development of an agricultural management program for date palm tree services, the application of quality control measures, and an increase in capacity building to reduce the cost of production;
- Make introducing the technology to the responsibility of the government; it cannot be left to farmers;
- Valorization of the date palm by-products (to generate more profit for the date palm producers).
- Polycarbonate projects should be targeted at areas with high levels of date production.

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8. REFERENCES

Al-Farsi M., Alasalvar C., Morris A. et al. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry* 53: 7592–7599.

Al-Marshudi A.S. (2002). Oman traditional date palms: production and improvement of date palms in Oman.

Tropiculture 20(4):203–209.

Al-Yahyai R. (2007). Improvement of date palm production in the Sultanate of Oman. Acta Horticulturae 736:337–343. Al-Yahyai, R., Khan, M.M. (2015). Date palm and perspective in Oman. Pages 207–240. In Date Palm Genetic Resources and Utilization: Volume 2: Asia and Europe (J.M. Al-Khayri, S.M. Jain, and D.V. Johnson eds).

Springer, Dordrecht, Heidelberg, New York, London.

Boz, I., and Akbay, C. (2005). Factors influencing the adoption of maize in Kahramanmaras province of Turkey.

Agricultural Economics, 33, 431-440.

Dhehibi, B., Aw-Hassan, A., Ben Salah, M., Al Raisi, Y., Al Bousaidi, I., Al Amri, S., Al Sobahi, S. (2016a). Economic comparison and evaluation of manual and liquid pollination methods of date palm trees in the Sultanate of Oman (varieties Fardh and Khalas). ICARDA Technical Report, 11p.

Dhehibi, B., Aw-Hassan, A., Ben Salah, M., Al Raisi, Y., Al Bousaidi, I., Al Amri, S., Al Shoaily, K. (2016b). Economic

evaluation of a polycarbonate drying house for date palm products. ICARDA Technical Report, 10p.

El Mardi, M.O., Esechie, H., AI-Kharousi, L.M., Abdelbasit, K.M. (2002). Effect of pollination method on changes in physical and chemical characteristics of date fruit during development. Agricultural Science 7(1):21–27.

Food and Agriculture Organization of the United Nations (FAO) (2011). FAOSTAT. Crop Production, Statistics

Division, FAO, Rome. http://www.faostat.fao.org.

Food and Agriculture Organization of the United Nations (FAO) (2014). FAOSTAT. Crop Production, Statistics

Division, FAO, Rome. http://www.faostat.fao.org.

Griliches, Z. (1957). Hybrid corn: An exploration in the economics of technological change. Econometrica, 25, 501-522.

Janjai S, Lamlert N, Intawee P, Mahayothee B, Bala B, Nagle M, et al. (2009). Experimental and simulated performance of a PV-ventilated solar greenhouse dryer for drying of peeled longan and banana. Sol Energy, 83, 1550–1565.

Janjai S, Intawee P, Kaewkiew J, Sritus C, Khamvongsa V. (2011) A large-scale solar greenhouse dryer using

polycarbonate cover: modeling and testing in a tropical environment of Lao People's Democratic Republic.

Renewable Energy, 36, 1053–1062.

King, R., and Rollins, T. (1995). Factors influencing the adoption of a nitrogen testing program. Journal of Extension,

33(4). Available on the World Wide Web: http://www.joe.org/joe/1995august/rb2.php.

Krueger, R.A. 2002. “Analysis: Systematic Analysis Process.” Website. Accessed March 10, 2008, at

www.tc.umn.edu/~rkrueger/focus_analysis.html.

Kuehne, G., Llewellyn R., Pannell, D., Wilkinson, R., Dolling, P., Ouzman, J. (2013). ADOPT: the Adoption and Diffusion Outcome Prediction Tool (Public Release Version 1.0, June 2013) [Computer software] Adelaide SA; CSIRO. Available from www.csiro.au/ADOPT

Marra, M., and Carlson, G. (1987). The role of farm size and resource constraints in the choice between risky technologies. Western Journal of Agricultural Economics, 12(2), 109-118.

Ministry of Agriculture and Fisheries (MAF) (2015). Annual report. MAF, Muscat, Sultanate of Oman.

Oman–Statistical Yearbook 2015. Issue 43. https://www.ncsi.gov.om/Elibrary/Pages/LibraryContentDetails.aspx?ItemID=lffQDcPJGNjEE5Xix4WK2g%3D%3D.

Prakash, O., Kumar, A. (2014). Solar greenhouse drying: a review. Renewable and Sustainable Energy Reviews. 29, 905–910. Rahm, M., and Huffman, W. (1984). The adoption of reduced tillage: The role of human capital and other variables.

American Journal of Agricultural Economics, 66(4), 405-413.

Rogers, E. (1995). Diffusion of innovations (4th ed.). New York: The Free Press.

Sangamithra, A., John Swamy, G., Prema, R.S., Priyavarshini, R., Chandrasekar, V., Sasikala, S. (2014). An overview of a polyhouse dryer. Renewable and Sustainable Energy Reviews, 40, 902-910.

Shahi, N.C., Khan, J.N, Lohani, U.C., Singh, A., Kumar, A. (2011). Development of polyhouse type solar dryer for

Kashmir valley. Journal of Food Science Technology, 48, 290–295.

Tables

Table 1. Predicted Adoption Levels of LPT at North and South Al Batinah – Sultanate of Oman

Predicted Peak Level and Time of LP Adoption	North Al Batinah Governorate	South Al Batinah Governorate
Predicted years to peak adoption	14.5	16.9
Predicted peak level of adoption	95%	95%
Predicted adoption level in 5 years from start	46.9%	35.8%
Predicted adoption level in 10 years from start	91.5%	85.8%

Source: Own elaboration from ADOPT (2017).
Note: Focus groups (# 12 farmers).

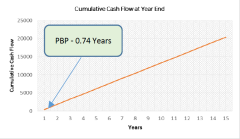
Table 2. Predicted Adoption Levels of PDH Technology at "South Al Batinah" Governorate – Sultanate of Oman

Predicted Peak Level and Time of PDH Adoption	South Al Batinah Governorate
Predicted years to peak adoption	20.9
Predicted peak level of adoption	95%
Predicted adoption level in 5 years from start	23.5%
Predicted adoption level in 10 years from start	72.9%

Source: Own elaboration from ADOPT (2017).
Note: Focus groups (# 10 farmers).

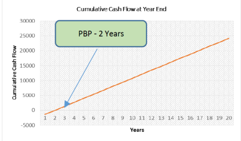
Figures

Figure 1: Cumulative Cash Flow at end of year (PBP when the small PDH subsidized)



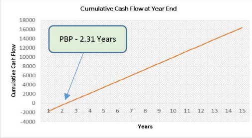
Source: Dhehibi et al., (2016).

Figure 2: Cumulative Cash Flow at end of year (PBP when the large PDH subsidized)



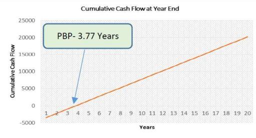
Source: Dhehibi et al., (2016).

Figure 3: Cumulative Cash Flow at end of year (PBP when the small PDH is not subsidized)



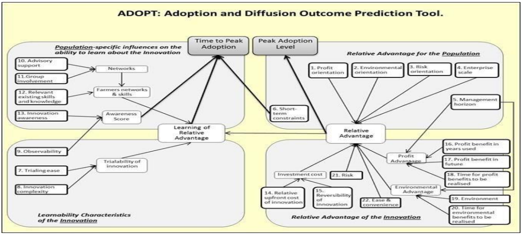
Source: Dhehibi et al., (2016).

Figure 4: Cumulative Cash Flow at end of year (PBP when the large PDH is not subsidized)



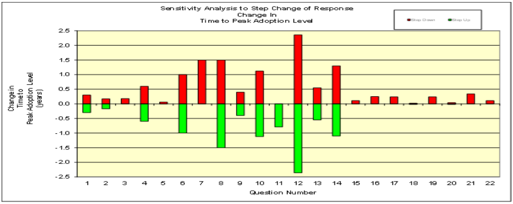
Source: Dhehibi et al., (2016).

Figure 5: Adoption and Diffusion Outcome Prediction Tool (ADOPT)



Source: http://aciar.gov.au/files/node/13992/adopt_a_tool_for_evaluating_adoptability_of_agric_94588.pdf.

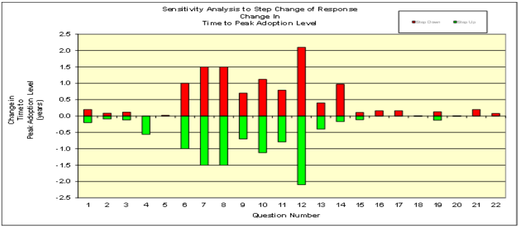
Figure 6: Sensitivity Analysis of Adoption Curve of LPT at "North Al Batinah" Governorate - Sultanate of Oman



Source: Own elaboration from ADOPT (2017).

Note 1: Red Column: Step Down; Green Column: Step Up. Note 2: Focus groups (# 12 farmers).

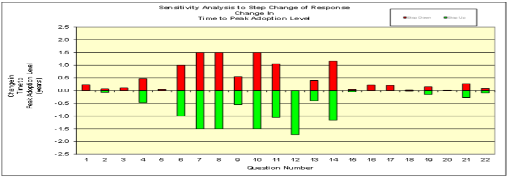
Figure 7: Sensitivity Analysis of Adoption Curve of LPT at "South Al Batinah" Governorate - Sultanate of Oman



Source: Own elaboration from ADOPT (2017).

Note 1: Red Column: Step Down; Green Column: Step Up. Note 2: Focus groups (# 12 farmers).

Figure 8: Sensitivity Analysis of Adoption Curve of PDH Dryer Technology at the “South Al Batinah” Governorate - Sultanate of Oman



Source: Own elaboration from ADOPT (2017).

Note 1: Red Column: Step Down; Green Column: Step Up. Note 2: Focus groups (# 10 farmers).

Drying dates using solar energy under polycarbonate house- new promising technology to dry dates in Oman

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Abstract

The project "Development of Sustainable Date Palm Production Systems" in GCC countries aims to use proper agro-management techniques, and to develop proper post-harvest techniques to reduce losses, and improve marketing. Drying dates by solar energy under Polycarbonate Drying House (PDH) is developed by the project. The PDH is a drying chamber covered by polycarbonate sheet equipped with exhaust fan. The polycarbonate sheet has superior properties in terms of transparency, transmissivity, anti-corrosion and tensile properties, tear-resistant, anti-puncture, water and moisture proof. Advantages of using PDH include: (1) Improved dates quality, (2) Avoiding contamination of dates by insects, dust, and rodents; (3) Accelerated drying process and (4) Reduced loss rates. Implementation of PDH has positive socioeconomic impacts on local income generation, food security and a sustainable date farming system. Results showed that Khsab dates dried from initial 70.09% to final moisture content of 11.3% in 8 days inside the PDH while required 13 days to decrease moisture from 70.09 to 21.45% under sun drying conditions. Economic evaluation of the PDH in Oman reveals the high profitability of the PDH, even when it is not subsidized by the government. At a real discount rate of 5%, the net present value (NPV) is positive and very high in all cases. The estimated Internal Rate Return (IRR) was higher than the current interest rate in the Oman, which could encourage both date palm growers and private investors to invest in PDH. Adoption and Diffusion Outcome Prediction Tool (ADOPT) was used to focus groups of date palm growers in Al-Batinah governorates in Oman to predicts the proportion of a target population that might adopt an innovation over time. The assessment of adoption of the PDH reveals that 95% of the farmers in Al-Batinah would adopt it after 21 years. However, the predicted adoption levels in 5 and 10 years from start are 23% and 73%, respectively.

Key words: Date palm, dates, Drying, DPH, Economic evaluation, ADOPT, Oman

INTRODUCTION

In Oman, date palm is the primary agricultural crop, and it constitutes 80% of all fruit crops produced and about 50 % of the total agricultural area in the country (FAO, 2013). Oman is the eighth largest producer of dates in the world with an average annual production of 260,000 tons per annum. There are approximately more than over seven million date palms and 250 cultivars in cultivation, primarily in the northern governorates of the sultanate. Only half of the dates produced are used for human consumption, with the other half being utilized primarily for animal feed or considered surplus and wasted (Al-Yahyai and Khan, 2015). The loss is high and estimated to more than 30% of the production.

Given the variability in the topographic and climatic growing conditions, the date palm production season extends from May to November, the longest season of the date production. In the North and South Al-Batinah governorates, major production is soft and semi-soft dates. For conservation drying of fruits is essential. Traditional way of drying date is made under the sun and dates are exposed to dust, birds and insects.

A series of experiments have been conducted to identify the appropriate technical specifications and dimensions of the drying houses to increase their efficiency. Drying dates inside plastic room was performed for the first time to ameliorate drying process. The quantity of dates to dry was limited and the colour was affected because limited aeration of the small plastic houses. Drying dates by solar energy under Polycarbonate Drying House (PDH) is developed by the project. The PDH is a drying chamber cover by polycarbonate sheet equipped with exhaust fan. The polycarbonate sheet has superior properties in terms of transparency, transmissivity, anti-corrosion, tensile properties, tear-resistant, anti-puncture, water and moisture proof.

The present paper present results of assessment in research station using one of the main important date palm cultivar (Khasab) compared with another line raised from seed. Economic evaluation of PDH with farmers in the Sultanate of Oman and prediction of adoption of the technology in the Omani conditions are studied.

MATERIALS AND METHODS

Dates of Khasab cultivar and dates of seed grown palm coded # 6 were entered in the chamber at Bisr and Rutab stages of maturity. Simultaneously, a representative sample were placed in well aerated spot on stainless steel mesh under the direct sun and temperature and humidity were monitored using weather station data loggers set to take a reading every 30 minutes. Polycarbonate chamber is manufactured locally and placed in the Agriculture Research Station, Rumais.

The dates' moisture was measured upon reception and after maturity when the fruits' weight stabilized. Data loggers (watch dog) supplied by Spectrum Technologies Inc., Aurora, USA was used to follow temperature inside and outside the polycarbonate house. Moisture balance supplied by KERN & Sohn, Baligen, Germany.

For technology economic evaluation, data were collected from two farmers in the Sultanate of Oman with two different polycarbonate drying house sizes, using rapid rural appraisal surveys with a direct interview method conducted at the same time as the survey. The information collected covered the capital cost, capacity of dryer, maintenance costs, lifespan of dryer, amount of subsidy received, and some technical information related to the condition of the fresh and dried dates. The data were used to assess and evaluate the economic significance of the polycarbonate dryer using the payback period method.

To evaluate a level of adoption of this technology, a focus group discussion (FGD) methodology was used to apply the ADOPT with group of date palm growers in North and South Al-Batinah in Oman. Streamlined 22 discussion questions around four categories of influences on adoption: characteristics of the innovation, characteristics of the target population, relative advantage of using the innovation and learning of the relative advantage of the innovation.

RESULTS AND DISCUSSIONS

Drying dates assessment in the research station

The dates drying process involves the loss of weight due to evaporation of moisture from the fruits which is assisted by high temperature and low humid moving air. The design of the Polycarbonate Drying House (PDH) (Fig. 1) is an attempt to provide optimal conditions to achieve drying requirements.

Data collected of the changes of temperature and humidity in 24 hours during the maturing process in and outside the DPH are depicted in (Fig. 2). The highest temperature recorded inside the DPH was 69.8ºC at 1:26 p.m. and the highest humidity was 62.7% at 4:56 a.m. while the highest humidity recorded outside was 99.9% and continued from 00.56 a.m. to 2:26 a.m. and the highest temperature was 41.7ºC.

Fruits of two cultivars (namely Khasab and a seed grown cultivar coded C6) are ripened at Bisr or Rutab stages (as they are naturally mixed in the bunches) and immediately moisture% is measured, and three samples (each weighed between 400-500g) are placed in stainless steel trays and entered in the PDH, the same was repeated for samples to be placed in the open air under the sun and all are monitored daily for changes in their weight.

The results obtained in this work showed that Khsab dates dried from initial moisture of 70.09 to final % moisture content of 11.3 in 8 days inside the PDH while required 13 days to decrease in moisture from 70.09 to 21.45 under sun drying conditions. The seed cultivar dried from initial moisture of 43.43 to final % moisture content of 15.8 in 8 days inside the PDH. The same time was required to decrease in moisture from 43.43 to 21.69 under sun drying conditions.

It was observed with Khasab cultivar (Fig. 3) that decrease in weight started rapidly from the first day and continued for four days in the PDH then stabilized. In the traditional drying the decrease was slower and continued for 12 days.

The decrease in weight was less prevalent in the C6 cultivar (Fig. 4), while decrease was steeper inside the PDH and prevailed in day 5 the difference between PDH drying and traditional was slight. However, due to higher humidity the samples placed outside started gaining weight after day 7 which is attributed to the hygroscopic nature of the dates.

Economic evaluation of PDH with farmers in Sultanate of Oman

PDH were evaluated economically based on Payback Period (PBP) (Nayak et al., 2012) under two scenarios: with and without governmental subsidies (Table 3, Fig. 4 and 5). Empirical findings reveal the high profitability of the polycarbonate drying system, even when it is not subsidized by the government. At a real discount rate of 5.1%, the Net Present Value (NPV) is positive and very high in all cases. Given this, the decision criterion states that an investment is usually acceptable if the NPV is positive (the investment is profitable). This criterion was also supported by both the Internal Rate Return (IRR) and the PBP criteria. The estimated IRR was higher than the current interest rate in the Sultanate, which could encourage both date palm growers and private investors to invest in polycarbonate drying houses. The PBP was found, in the worst case scenario, to be 3.77 years, which is relatively short considering the life of the system (15-20 years). This suggests that investment or action costs in this dryer system are recovered quickly reducing the risk involved in the investment.

Prediction of adoption of the technology

The ADOPT tool predicts the proportion of a target population that might adopt an innovation over time. The assessment of PDH technology (Table 4, Fig. 6 and 7) reveal that 95% of Al Batinah community would adopt the innovations after 20.9 years. However, the predicted adoption levels in 5 and 10 years from start are 23.5% and 72.9%, respectively. These results are expected since the upfront cost of investment is quite high while the economic viability of this technology make the evidence of its profitability. Results suggest that triability of the innovation on a limited basis before a decision is made to adopt it on a larger scale, the perception and evaluation of the PDH technique. The size of the up-front cost of the investment relative to the potential annual benefit from using the innovation are the driving adoption factors for the PDH technology in the target area.

CONCLUSION

The PDH was proved to be an efficient method to mature dates by providing optimal conditions to assist the dates drying process. The drying in the PDH reduce the time of drying date to 4 days, instead 8-12 days by the traditional drying way in addition to protecting the dates from different elements such as high humidity, dust and attacks of insects and birds.

Based on economic indicators (NPV, IRR and PBP) the investment is highly profitable. The investment is usually acceptable if the NPV is positive.

The results of predict of the adoption of the PDH indicate that to meet the technical, economic, and socioeconomic requirements, there is a need for a greater political and institutional input into polycarbonate drying houses projects. In particular, there is a need to design and develop alternative policy instruments (other than subsidies) and institutions for extension, technical assistance, training, and credit services that will facilitate adoption of this technology.

REFERENCES

Al-Yahyai, R., Khan, M.M. (2015). Date palm and perspective in Oman. Pages 207–240. In Date Palm Genetic Resources and Utilization: Volume 2: Asia and Europe (J.M. Al-Khayri, S.M. Jain, and D.V. Johnson eds). Springer, Dordrecht, Heidelberg, New York, London.

Dhehibi B., A. Aw-Hassan, M. Ben Salah, Y. Al Raisi, I. Al Bousaidi, S. Al Amri and S. and K. Al –Shoaily. 2016. Economic Evaluation of a Polycarbonate Drying House for Date Palm Products. Technical Report. Development of sustainable date palm production systems in the GCC countries of the Arabian Peninsula. ICARDA. April 2016. 12 pages.

Dhehibi B., M. Ben Salah, A. Frija, A. Aw-Hassan, Y. Al Raisi, I. Al Bousaidi, S. Al Amri, S. Sobahi, and K. Al Shoaihli. 2018. Predicting Farmers' Willingness to Adopt Liquid Pollination and Polycarbonate Drying Technologies: A Case Study from the Date Palm Growers in the Sultanate of Oman. Presented to Sustainable Agriculture Research Journal. February 2018.

Nayak, S., Z. Naaz, P. Yadav and R. Chaudhary. 2012. Economic analysis of hybrid photovoltaic-thermal (PVT) integrated solar dryer. *International Journal of Engineering Inventions* 1(11): 21-27. February 2018.

International Center for Agricultural Research in Dry Areas (ICARDA). 2015. Report of the project Development of Sustainable Date Palm Production Systems in Gulf Cooperation Council Countries.

Ministry of Agriculture and Fisheries (MAF) (2017). Annual report. MAF, Muscat, Sultanate of Oman.

Tables:

Table 1. Moisture (%) of Khasab dates before and after maturity

Sample	Initial	Final
Inside the PDH	70.09	11.3
Outside	70.09	21.45

Table 1. Moisture (%) of C6 dates before and after maturity

Sample	Initial	Final
Inside the PDH	43.43	15.8
Outside	43.43	21.62

Table 3. Computation of economic indicators and financial indicators (NPV, IRR, and PBP) of the PDH

Items	Subsidized dryer - FIRR	No subsidy for dryer - EIRR
Fresh dates (OMR/kg)	0.8	0.8
Dried dates (OMR/kg)	1.2	1.2
Product net weight (kg/kg of fresh date)	0.75	0.75
Capacity of dryer (kg/year)	6000	6000
Cost of fresh materials	6000*0.8*0.65 3120	6000*0.8*0.65 3120
Labor and maintenance costs (OMR)	300	300
Electricity costs (OMR)	36	36
Cost of insurance (OMR)	0.0	0.0
Total cost of fresh materials	3456	3456
Capital cost of dryer (OMR)	1000	3000
Life of dryer (years)	15	15
Depreciation (OMR/year)	66.66	200
Total revenue (OMR)	(6000*0.75*0.75*1.2)+(6000*0.75*0.25*0.8) 4950	(6000*0.75*0.75*1.2) +(6000*0.75*0.25*0.8) 4950
Total cost (OMR/year)	4522.66	6656
Net income (OMR/year)	427.34	-1706
Financial Indicators		
Net present value (NPV)	12,764.184	7486.52
Financial and economic internal rates of return (FIRR, EIRR)	Very high (+100%)	76%
Payback period (PBP, years)	0.7	2.31

Source: Dhehibi et al. (2017)

Table 4. Predicted Adoption Levels of PDH Technology at "South Al Batinah" Governorate – Sultanate of Oman

Predicted Peak Level and Time of PDH Adoption	Al Batinah Governorate
Predicted years to peak adoption	20.9
Predicted peak level of adoption	95%
Predicted adoption level in 5 years from start	23.5%
Predicted adoption level in 10 years from start	72.9%

Source: Dhehibi et al (2017)

Figures

Fig. 1. Polycarbonate Drying House (PDH)

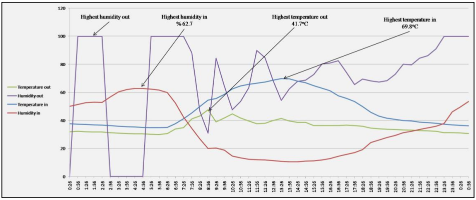


Fig. 2. Changes in temperature and humidity in and out of the PDH

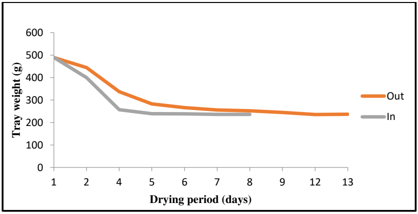


Fig. 3. Changes in Khasab dates weight upon maturity

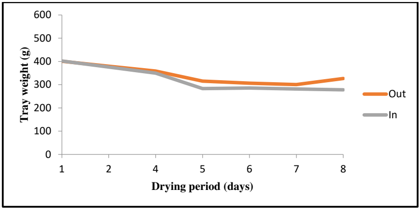
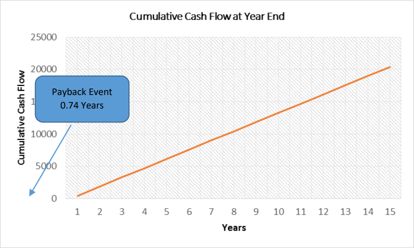
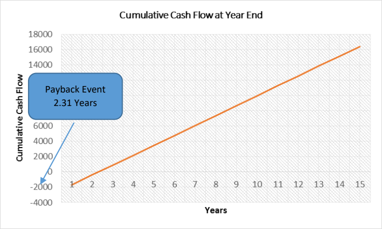


Fig. 4. Changes in C6 date variety weight upon maturity



Source: Dhehibi et al. (2016)

Fig. 5. Cumulative cash flow at year end (PBP) when the PDH is subsidized

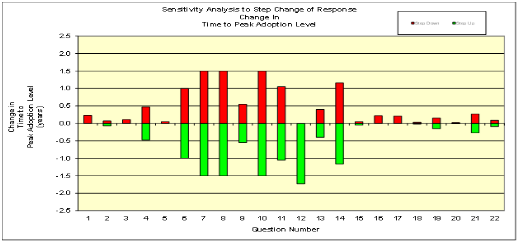


Source: Dhehibi et al. (2016)

Fig. 6. Cumulative cash flow at year end (PBP) when the PDH is not subsidized

Source: Dhehibi et al. (2017)

Fig. 7. Predicted Adoption Curve of PDH Dryer Technology at the "South Al Batinah" Governorate - Sultanate of Oman



Source: Dhehibi et al. (2017)

Fig. 8. Sensitivity Analysis of Adoption Curve of PDH Dryer Technology at the "South Al Batinah" Governorate - Sultanate of Oman

Liquid pollination technology: A new technology to Ameliorate date palm pollination and facilitate date palm field operations

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Abstract

Pollination of date palm is normally carried out by hand in almost all date palm groves in Oman. Globally, farmers use various hand pollination techniques. Dry pollination is used utilizing hand and motorized dusters with no negative effect on fruit yield. However, larger pollen volumes are used with the dusters and uneven distribution of pollen grains is found when the palms are tall. With Liquid Pollination (LP), female trees flowers are sprayed with pollen grains suspended in water using knapsack or motorized sprayers. A series of field trials revealed that LP resulted in fruit set and other fruit quality parameters similar to those obtained by traditional manual pollination (TMP). In addition, it contributes to saving time, reducing cost and reduces the risk of climbing accidents to laborers.

LP was evaluated economically versus TMP for Fardh cultivar based on the data collected from researchers from the Date Palm Research Center in Wadi Quriyat in Oman. The reduction in pollination cost using LP in comparison with TMP is about 89% and, consequently, a reduction in the total variable costs per hectare against those for manual pollination of about 42-56%. Economic indicators showed that LP will be highly profitable for Omani farmers. The Net Return (NT) using liquid pollination was very high (+ 674%). The benefit-cost ratio (BCR) is three times higher when using liquid pollination. ADOPT (Adoption and Diffusion Outcome Prediction Tool) tool was used to focus groups of date palm growers in Oman to predict the proportion of a target population that might adopt an innovation over time. The empirical findings obtained from the LP assessment indicate that peak adoption rate for the technology in Al-Batinah is predicted to be 95% after a period of 14-16 years. The predicted adoption level in 5 years and 10 years from start is expected to be 35-47% and 86-91%, respectively.

Key words: Date palm, pollination, LP, Economic evaluation, ADOPT, Oman.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is the widest spread crop and attain great cultural, social and religious importance in the Sultanate of Oman. The total number of date palm trees in Oman is estimated at about 8 million which occupies about 49% of total area under cultivation and 85% of the area cultivated by fruit trees. Currently, Oman is the eighth largest world producer of dates with a total production reaching 361,000 ton (MAF, 2017). The dates are mainly harvested for fresh fruit consumption. However, alternative goods, such as date syrup, date sugar, and other by-products, can also be found in the local markets.

Oman has diverse topographical and climatic eco-regions that allow for the cultivation of various types of date palm cultivars, particularly in the northern coastal and interior regions. There are approximately 180 female and 48 male cultivated varieties. Given the variability in the topographic and climatic growing conditions, the date palm production season extends from May to November, the longest season of any date-producing country. According to Al-Marshudi (2002) and Al-Yahyai and Khan (2015), the yield of the date palm is considered to be low (40-80 kg/tree) compared to the yields in neighboring countries (i.e. Saudi Arabia and UAE). This low yield is a result of traditional management, lack of farmer know-how, high infestation by several pests, limited field expansion because date growing regions are fully dependent on groundwater extraction for irrigation, in addition to logistic problems, including an insufficient number of skilled laborers and underdeveloped facilities (transport, storage, market outlets, and large processing factories).

The date palm, being a dioecious plant, need to be pollinated. Pollination is performed traditionally in Oman and elsewhere by climbing the female trees to insert the male flower strands into the female spathe. This is laborious, time consuming and a risky operation to farm labors. A number of successful studies have been done in Oman and other countries to use machine for pollination in which farmers can perform the operation from the ground quickly and easily. Al-Bakr (1972) mentioned that pollination machine was introduced to the USA as early as 1952 to dust the pollen from ground level. The operation was completed in just few hours as compared to as long as two days to manually pollinate an area of two feddans. El Mardi, et al. (2002) found that the fruit set, yield and fruit quality in mechanical pollination was similar to that of traditional method which was supported by Brown et al. (1969) and Shabana et al., (1985) in: Dhehibi et al. 2018).

As a result, there is a great need and potential for improving the current date palm growing practices. In the current project a novel approach was used to pollinate female date palm flowers using pollen grains suspended in water (Liquid Pollination = LP) which is sprayed from ground level using motorized sprayers. Technical and economical aspects of adopting this technique were studied.

METHODOLOGY

A number of consecutive field experiments were conducted by the researchers of date palm horticulture research section, date palm research center (DPRC) starting from 2003/2004 season. The aim of the experiments was to standardize the LP technique by assessing various pollen grain concentrations using different varieties in different agro-ecological zones in Oman. Subsequently, the technology was demonstrated at farmers' field level. Following the success of LP, it was disseminated to the various agricultural governorates through the extension specialists after conducting a series of workshops for training of the subject matter specialists. LP technique was disseminated to the various Gulf Cooperation Council (GCC) countries through the regional project entitled "Development of Sustainable Date Palm Production Systems in the GCC Countries of the Arabian Peninsula" coordinated by ICARDA. Furthermore, eeconomic comparison and evaluation of manual and liquid pollination methods of date palm trees was performed in the Sultanate of Oman for the varieties Fardh and Khalas by the project experts. A Case Study was performed from the date palm growers in the Sultanate of Oman for predicting farmers' willingness to adopt LP technique.

RESULTS AND DISCUSSION

The series of field trials conducted by DPRC revealed that LP resulted in fruit set and other fruit quality parameters similar to those obtained by TMP. The study conducted on Fardh variety proved that pollen grain suspension spray at 0.5g/L water resulted in fruit set (75.9 %) compared to the TMP (71.3%) with no statistical significant difference between the treatments ($P>0.05$). LP also resulted in as good fruit weight and total yield as the TMP with no significant difference. Increasing pollen grain concentration even up to triple the concentration, did not improve the studied parameters. However, significant reduction ($P>0.05$) on fruit set was observed on Barni variety fruit set using LP at 0.5g/L water (66.0%) when compared to TMP. However, the total yield (Kg/palm) was not affected (106.9 Kg/palm for LP V.S. 102.9 Kg/palm for TMP). Moreover, the fruit weight at the full mature Tamr stage was significantly increased ($P>0.05$) with LP (4.4g) compared to TMP (3.8g) (Table 1). Similar results were obtained with the variety Jabri (MAF, 2005/2006). This is considered as an advantage of LP resulting in less competition of the fruits for water and nutrients which plays as natural thinning of fruits.

Economic evaluation of LP with farmers in Sultanate of Oman

Economic assessment of the liquid pollination technology was performed from two perspectives, i.e, research and the end users (farmers) for Fardh as commercial variety in Oman. Affordability and profitability were analyzed using a partial budget analysis tool and disaggregated data gathered from researchers and farmers, using rapid rural appraisal surveys.

Results (Table 2) indicates a reduction in pollination cost using liquid pollination in comparison to that for manual pollination of about 89.05% and, consequently, a reduction in the total variable costs per hectare against those for manual pollination of about 56.48%. Moreover, the analysis reveals a total reduction in the variable costs of OMR 1273.95 from using LP. This reduction in total variable costs results from an increase in the net revenue over that resulting from manual pollination of OMR 2593.95/ha. The economic indicators (Table 2) show also the clear profitability of using liquid pollination where the percentage change in net returns is very high (+ 674.71%). The benefit-cost ration (BCR) is three times higher when using liquid pollination. Thus, with an internal rate of return with 12.04 and higher BCR, we can conclude how profitable it is for Omani farmers to use liquid pollination.

Analyze of Adoption of LP in Sultanate of Oman

The adoption of new agricultural technologies has generally been found to be a function of farm and farmer characteristics and specific features of the particular technology (Feder et *al*., 1985; Marra and Carlson, 1987; Rahm and Huffman, 1984 in Dhehibi et al. 2018). A considerable set of literature was developed regarding factors that influence the adoption of new technologies by farmers through use of innovation theory (Feder et *al*., 1985; Griliches, 1957, and Rogers, 1995 in Dhehibi et al. 2018).

Rogers (1995) identified five characteristics of an innovation that affect an individual's adoption decision:

- Relative advantage: how the innovation is better than existing technology;
- Compatibility: the degree to which an innovation is seen as consistent with existing experiences, needs, and beliefs of adopters;
- Complexity: how difficult the innovation is to understand and use;
- Trialability: the degree to which the innovation may be used on a limited basis;
- Observability: the degree to which the results of an innovation are visible to others.

As the relative advantage, compatibility, complexity, trialability, and observability of liquid pollination have caused more farmers to adopt it in the Sultanate of Oman, in particular, and in the GCC countries in general. We can consider the adoption of LP as an innovation. The utilization and critical mass adoption of such technologies is an important prerequisite for agricultural development, particularly for the date palm producing countries in the Arabian Peninsula.

The results obtained about the LP technology adoption (Figure 1) in North Al-Batinah region as example (Dhehibi et al. 2018), indicate that sustainable increases in productivity of date palm in the Sultanate of Oman can be achieved if farmers are encouraged to adopt the liquid pollination technology. The peak adoption rate for liquid pollination technology in the "North Al-Batinah" is predicted to be 95% after a period of 14.5 years. The predicted adoption level in 5 years and 10 years from start is expected to be 46.9% and 91.5%, respectively. However, the adoption of such technology needs to be accompanied by a supporting extension system and an enabling political environment to ensure the scaling-up and widespread use of this promising and profitable technology.

CONCLUSION

Extension of LP as new technology, economic evaluation and analyze of adoption in Sultanate of Oman shows the following advantages and constraints regarding the use of this technology:

The main advantages of using liquid pollination are:

- Saves time and effort (reducing labor cost and improving the effectiveness and productivity of the labor used)
- Reduces the quantity of pollen needed
- Reduces labor and pollen costs
- Reduces the risk low fruit set by pollinating during the peak period of flowering

- Improves the quality of the fruits and consequently the profitability of the varieties intended for export
- Improves the fruit setting percentage
- Contributes to reducing harvesting losses
- Reduces the risk of climbing accidents to laborers.

Liquid pollination have also some constraints:

- The pollination extraction device is expensive (around OMR3500), which small-scale farmers cannot afford,
- Limited number of date palm trees per farmer (the investment in the pollination extraction device is not profitable),
- Resistance of farmers to adopting the new technology and to changing their practices (farmers are accustomed to the old technology of hand pollination),
- Lack of specialized extension staff for the date palm,
- No interest from the younger generation in date palm production.

Some suggested policy options for accelerating the adoption process and scaling-up the use of liquid pollination technology include:

- Development of an agricultural management program for date palm tree services, the application of quality control measures, and an increase in capacity building to reduce the cost of production,
- Creation of private companies to carry out and monitor the liquid pollination operations with support from the government,
- Enhancing the extension services (more and specialized extension agents) and the development of an effective extension service for Omani date palm growers,
- Reinstatement of the subsidy system in the sector,
- Enhancing the awareness of farmers regarding the profitability of using this technology in comparison to the manual pollination method.

REFERENCES

Al-Bakr A., 1972. The Date Palm. Its Past and Present Status. Alani Press, Baghdad.

Al-Marshudi A.S. (2002). Oman traditional date palms: production and improvement of date palms in Oman. Tropiculturae 20 (4):203–209.

Al-Yahyai, R., Khan, M.M. (2015). Date palm and perspective in Oman. Pages 207– 240. In Date Palm Genetic Resources and Utilization: Volume 2: Asia and Europe (J.M. Al-Khayri, S.M. Jain, and D.V. Johnson eds). Springer, Dordrecht, Heidelberg, New York, London.

Ben Salah M. and Y. Al-Raisi. 2017. Liquid pollination: New field technique to enhance the date palm production. 2nd National Conference on Agriculture and Fisheries Research (Research for Sustainable Development). 3-4 April 2017. Sultan Qaboos University, Muscat-Oman.

Dhehibi B., A. Aw-Hassan, M. Ben Salah, Y. Al Raisi, I. Al Bousaidi, S. Al Amri and S. Al Sabahi, 2016. Economic Comparison and Evaluation between Manual and Liquid Pollination Methods of Date Palm Trees in the Sultanate of Oman (Varieties Fardh and Khalaas). Technical Report. Development of sustainable date palm production systems in the GCC countries of the Arabian Peninsula. ICARDA. April 2016. 12 pages.

Dhehibi B., M. Ben Salah, A. Frija, A. Aw-Hassan, Y. Al Raisi, I. Al Bousaidi, S. Al Amri, S. Sobahi, and K. Al Shoaihli, 2018. Predicting Farmers' Willingness to Adopt

Liquid Pollination and Polycarbonate Drying Technologies: A Case Study from the Date Palm Growers in the Sultanate of Oman. Presented to Sustainable Agriculture Research Journal. February 2018.

El Mardi, M.O., Esechie, H., AI-Kharousi, L.M., Abdelbasit, K.M, 2002. Effect of pollination method on changes in physical and chemical characteristics of date fruit during development. Agricultural Science 7(1):21–27.

Rogers, E., 1995. Diffusion of innovations (4th ed.). New York: The Free Press.

International Center for Agricultural Research in Dry Areas (ICARDA), 2015. Report of the project Development of Sustainable Date Palm Production Systems in Gulf Cooperation Council Countries.

MAF, 2005/2006. Biennial Agricultural Research Report 2005/2006. Directorate General of Agriculture and Livestock Research, Ministry of Agriculture and Fisheries, Muscat,Sultanate of Oman, pp339-357.

MAF, 2017. Date Production in Oman 2017. Directorate General of planning and investment, Ministry of Agriculture and Fisheries, Muscat, Sultanate of Oman.

Tables

Table 1. Fruit set, total yield and fruit weight at Tamr stage of Barni date palm variety as affected by Liquid pollination (LP) compared to traditional manual pollination (TMP) during 2004/2005 seasons

Season	**First season**			**Second season**		
Treatment	Fruit set (%)	Total yield (Kg/palm)	Fruit weight (g)	Fruit set (%)	Total yield (Kg/palm)	Fruit weight (g)
Control (TMP)	83.1	102.9	3.8	77.3	63.4	5.6
LP (0.1 g/L)	58.7	85.6	5.4	57.4	68.9	6.7
LP (0.3 g/L)	68.5	102.2	4.8	52.1	52.5	7.4
LP (0.5 g/L)	66.0	106.9	4.4	62.2	60.2	7.0
LSD 5%	8.3	8.3	0.4	8.3	8.3	0.4

Source: (MAF, 2005/2006).

Table 2. Partial budget analysis for using liquid pollination with the date palm variety *Fardh* in the Sultanate of Oman

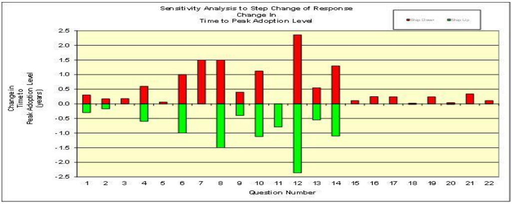
Variable	**With technology option (liquid pollination)**	**Without technology option (manual pollination)**
Yield (kg/tree) [(a)]	40	40
Number of date palm trees/ha	165	165
Yield (kg/ha)	6,600	6,600
Price (OMR/kg) [(b)]	0.6	0.4
Total value of production (OMR/ha)	**3,960**	**2,640**
Cost of pollen (OMR/ha)	125	935.55
Cost of device (OMR/ha)	11.6	0
Labor cost for pollination (OMR)	20	495
Total cost of pollination (OMR/ha) [(c)]	156.6	1,430.55
Other costs (irrigation, fertilization, pruning, thinning, harvesting, and post-harvest handling) (OMR/ha) [(d)]	825	825
Total variable costs (OMR/ha)	**981.60**	**2,255.55**
Net revenue (OMR/ha)	**2,978.40**	**384.45**

Economic indicators (1)		
Reduction of pollination cost per ha over manual pollination (%)	89.05	
Reduction of total variable costs per ha over manual pollination (%)	56.48	
Variable costs between the two technologies (OMR/ha)	- 1,273.95	
Increased net revenue over manual pollination (OMR/ha)	2,593.95	
Economic indicators (2)		
Net returns (NR)	**2,978.40**	**384.45**
Change in net returns (NR) (%)	674.71	
Change in total costs (TC) (%)	56.48	
Internal rate of return (IRR)	12.04	
Benefit-cost ratio (BCR)	4.03	1.17

Source: Dhehibi et al. 2016

Figures

Fig. 1. Sensitivity Analysis of Adoption Curve of Liquid Pollination Technology at "North Al Batinah" Governorate - Sultanate of Oman



Source: Dhehibi et al. 2018

Antioxidant and Acetylcholinesterase Inhibitory Activitiesand Phytochemical Analysis of Extracts from *Phoenix dactylifera* (Arecaceae)

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Abstract

Phoenix dactylifera **parts (fruits, leaves, pollen) have traditionally been used for treatment of a range of ailments such as diabetes, hypertension, atherosclerosis, cancer and infertility. In the present study, the antioxidant and acetylcholinesterase inhibitory activities of extracts from leaves and pollen of *P. dactylifera* were investigated by 2,2'-diphenyl-1-picrylhydrazyl(DPPH) and acetylcholinesterase (AChE) assays. Phytochemical analysis was made by TLC using authenticated standards. Dried leaf and pollen were extracted successively by dichloromethane and methanol, respectively. The hot-water and polyphenol extracts were also performed. All the extracts from the different parts of the plant showed antioxidant activity. The methanol leaf extract was the most active extract with the $IC_{50}$of 13.48 ± 0.18 µg/ml. In bioautography assay, the methanol leaf extract was also the most active showing several active zones. Only the polyphenol and methanol extracts from leaves showed AChE inhibitory activity with $IC_{50}$of 310.58 ± 0.74 and 382.05 ± 10.68 µg/ml, respectively. Two flavonoids (flavan and rutin) were identified by TLC analysis. These results suggested that *P. dactylifera* can be used in the treatment of neurodegenerative disorders such as Alzheimer and as sources of natural antioxidants in food industry.**

Keywords: bioautography, phenolic compounds, flavonoids, male leaf and pollen extracts

INTRODUCTION

Phoenix dactylifera L.is distributed in arid and semi-arid regions of Africa, Asia, and in Mediterranean climateregions of Europe.In Algeria, date palm is cultivated in the northern Sahara; themain date palm areas are located in the provinces of Biskra, El Oued, Adrar, Ghardaia and Ouargla(Selmani et al.,2017).

The fruit of the date palm is considered to be an important fruit for the population living in the Algerian Sahara. It is considered a vital component of their daily diet. This fruit has great importance from nutritional and economic points of view (Mansouri et al.,2005).

It has traditionally been used in Algeria for diarrhea, injuries, constipation, hypertension, edema, dizziness, gastric ulcers, easy delivery, hemorrhage, cancer, pain of sore, blood sugar, skin healing, broken arms, legs or back and elderly, brown spots on the skin and bites of scorpions. The pollen was used for male and female sterility,the leaves were used for cough and sore throat, and the seeds were used for weakness, gout disease and lactation (Selmani et al.,2007).

Several compounds have been characterized in *P. dactylifera* from Algeria i.e. gallic acid, chlorogenic acid,caffeic acid, coumaric acid, cinnamic acid,ferulic acid, sinapic acid, coumaroylquinic acid, vanillin, rutin, flavanone, flavonol andxantoxylin (Mansouri et al.,2005; Zohra et al.,2016).

Numerousbiologicalactivitieswere highlighted from various extracts of *P. dactylifera* from Algeria, such as anti-inflammatory, antioxidant, anti-amylase, antifungal and antimutagenic(Boulenouar et al.,2011; Eddine et al.,2014a,b; Haimoud et al.,2016).

The aim of this study was to evaluate the antioxidant and anti-acetylcholinesteraseactivities, and to determine the phenolic profile of different extracts from leaves and pollen of*P. dactylifera*. This study represents the first report characterizing the acetylcholinesterase (AChE) inhibitory activity of *P. dactylifera* male leaf and pollen.

MATERIALS AND METHODS

Chemicals and Reagents

Chloroform, dichloromethane, ethyl acetate, ethanol, methanol, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), quercetin, ascorbic acid, trolox, butylatedhydroxytoluene (BHT), hydrochloric acid, acetylcholinesterase from electric eel (EC 3.1.1.7, type V-S, lyophilized powder, 827 U/mg solid, 1256 U/mg protein), acetylthiocholine iodide (ATChI), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), galanthamine hydrobromide, tris, and bovine serum albumin (BSA)were purchased from Sigma (St. Louis, USA). Two different buffer systems were used (buffer A: 50 mMtris-HCl, pH 8 containing 0.1% BSA and buffer B: 50 mMtris-HCl, pH 8 containing 0.1 M NaCl and 0.02 $MMgCl_2{\cdot}6H_2O$). TLC silica gel 60 F_{254} aluminium plates were purchased from Merck (Darmstadt, Germany). All remaining chemicals used were of analytical grade.

Plant Material

Male leaves and pollen were collected in february 2017 from Biskra, Algeria. Botanical identification of the plant was conducted by one of the author (Prof. Malika Bennaceur). A voucher specimen of the plant (OUE.2017.1) was deposited in the collections of the laboratory of the first author. The plant materials were dried in a well-ventilated room at a temperature of 30 °C and stored in the dark until use.

Extraction

The male leaf and pollen dried powder (200 g) each was extracted successively with dichloromethane and methanol (1200 ml) at room temperature for 24 h three times. All extracts were filtered through a filter paper. The organic solvent was removed under reduced pressure at 40 °C, to yield dichloromethane and methanol extracts, respectively.

For aqueous extract, dried powder of male leafand pollen (10 g) was extracted three times under reflux by distilled water (100 ml) for 30 min. The extracts were filtered through filter paper, combined, and lyophilized to afford the aqueous extract.

For extraction of polyphenols, 10g of dry powder of male leaf and pollen was extracted three times under reflux by methanol 80% (100 ml) for 30 min. Then, the extracts were treated with HCl 2N (10 ml) solution. The organic phase was separated by liquid-liquid extraction with ethyl acetate (50 ml). This procedure was repeated 3 times. All the ethyl acetate extracts were mixed and evaporated at 40 °C.

Phytochemical Analysis

Phytochemical analysis was performed according to Wagner and Bladt (1996) to detect the presence of phenolic compounds.

Antioxidant Activity

1. TLC Bioautography Assay. Bioautographic evaluation was conducted to check the antioxidant activity ofextractsof male leaf and pollen from *P. dactylifera* on TLC silica gel60 F254 aluminium plates. In the assay, DPPH react with antioxidants andproduce colorless 2,2′-phenyl-1-picrylhydrazine. The samples were applied at25 μg. Quercetin was used as the positive control (1 μg). The developed airdriedplate was sprayed with methanolic solution of 0.2% DPPH and theplate was again air-dried. After 30 min, yellow spots were observed againstpurple background indicating antioxidant capacity (Cuendet et al., 1997).

2. Spectrophotometric Assay

DPPH radical scavenging activity was measured as described byCavin et al.(1998).Methanolic solution (5 μL) of each sample at five different concentrationswas added to 915 μL of methanol, and then 200 μL of DPPH solution wasadded (0.022% in methanol). The mixture was incubated at room temperaturein the dark and the absorbance of the reaction mixture was measured at517 nm after 30 min, against a blank of methanol without DPPH. The DPPHsolution without sample solution was used as control. Ascorbic acid, quercetin,trolox and BHT were used as reference compounds. Scavengingactivity was determined by the following equation:

% scavenging activity = $100\times(A_{control}-A_{sample})/A_{control}$

The percentage of scavenging activity was then plotted against the antioxidantconcentration to obtain the amount of antioxidant necessary todecrease the initial solution of DPPH by 50% (IC_{50}). Each experiment wasperformed in three replicates.

AChEInhibitory Assay

AChE was assayed according to the principle of hydrolysis of ATChI bymonitoring the formation of 5-thio-2-nitrobenzoate spectrometrically at412 nm (Ellman et al.,1961) and was conducted as described byIngkaninan et al. (2000)with slight modification.The reaction mixture contained 295 μL of buffer A, 100 μL of 0.226 U/mlAChE in buffer A, 5 μL of extracts or galanthamine (as positive control) inmethanol at five different concentrations or methanol (as negative control),and 500 μL of 3 mM DTNB in buffer B, which were mixedand incubated for15 min at 37°C. The reaction was then initiated in cuvettes by addition of100 μL of 15 mMATChI in water; and after the cuvettes were shaken for 5 s,the changes in absorbance were measured at 412 nm every 20 s for 4 minwith UV-visible spectrophotometer. All the reactions were performed intriplicate and the initial rates were measured as the rate of change inabsorbance/time. Percentage of inhibition of AChE was determined using the formula $1-(RR_{sample}/RR_{control})\times100$, where RR_{sample} is the rate ofreaction of sample and $RR_{control}$ is that of the negative control. The AChEinhibitory activity was expressed in terms of the IC_{50} value (sample concentrationrequired to inhibit the hydrolysis of the substrate by 50%).

Statistical Analysis

All analyses were carried out in triplicates. Data were presented as mean ±SEM. Data were analyzed by one-way analysis of variance followed byTukey's and Student-Newman-Keuls post hoc test for multiple comparisons ($P < 0.05$; SPSS V20 software).

RESULTS AND DISCUSSION

The percentage of extraction yields were 6.54% (w/w) for dichloromethane extract of leaves, 2.1% (w/w) for dichloromethane extract of pollen, 14.37% (w/w) for methanolic extract of leaves, 17.71% (w/w) for methanolic extract of pollen, 8.71% (w/w) for aqueous extract of leaves, 25.33% (w/w) for aqueous extract of pollen, 1.96% (w/w) for phenolic extract of leaves and 2.43% (w/w) for phenolic extract of pollen (Table 1).

TLC analysis of extracts revealed the presence of rutin and flavan in methanolic extract of leaves and dichloromethane extract of pollen, respectively. The identification was supported by the results of TLC with authentic standard compounds showingthesameRfandfluorescence(Fig. 1).

The antioxidant activity of extracts was tested on TLC using DPPH as the detecting reagent. All extracts of *P. dactylifera* at 25 µg showed positive zones with a purple background on TLC (Fig. 2). The dichloromethane extract of leaves was inactive. The positive control quercetin showed positive zone.

The antioxidant activity obtained by the DPPH assay for *P. dactylifera* extracts are shown in Table 1. The different extracts showed DPPH radical-scavenging activity in a concentration-dependent manner. Among extracts tested, the best results were obtained with methanolic extractof leaves with IC_{50} valueof 13.48 ± 0.18µg/ml. The DPPH radical scavenging activities were in following order: methanolic extract of leaves > phenolic extract of leaves >phenolic extract of pollen >aqueous extract of leaves > dichloromethane extract of leaves >dichloromethane extract of pollen >aqueous extract of pollen > methanolic extract of pollen. All the tested positive controls were more active than the extracts. Also, at previous studies, the DPPH scavenging potentials were reported for some extracts from *P. dactylifera*, such as leaflets (IC_{50}; 13.15 µg/ml), seed (93.80% at750 µg/ml), and coffee (42.69%at 750 µg/ml) (Bennaceur et al., 2010;Sekeroglu et al., 2012).

The spectrophotometric assay of AChE inhibitory activity of extracts (Table 1), showed activity for polyphenol extract of leaves (IC_{50}value of 310.58 ± 0.74 µg/ml) and methanolic extractof leaves (IC_{50}value of 382.05 ± 10.68 µg/ml). The other extracts were inactive in the test.This activity was considerably lower than that of the pharmacological drugs used to treat neurological diseases, such as galanthamine (IC_{50} of 0.30±0.0021 µg/ml). The flavonoid rutin, identified in methanolic extract of leaves has been demonstrated to be AChE inhibitors (Pinho et al., 2013), may has been responsible for this effect. However, the active extracts represent a new natural source of AChE inhibitors. It should be noted also that it is not a purified extracts.The inhibition of AChE by the seed extract of *P. dactylifera* was tested, showing similar activity to polyphenol and methanolicextracts of leaves of *P. dactylifera*, while coffee extract was less active (Sekeroglu et al.,2012). The results are very promising, because until this time AChE inhibitory activity of *P. dactylifera* has not been reported for leaf, as far as we know.

The results obtained from this study indicate that some extracts of *P. dactylifera*can serve as a potential source of naturalantioxidantsandacetylcholinesterase inhibitors, which could be suitable for food and pharmaceutical applications. The bioactive compounds should be separated and purified and its safety should be determined to investigate potential use. Further studies of structure-bioactivity relationship are also required.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Bennaceur, M., Bengag, A., Marouf, A. and Bouguedoura, N. 2010.Phytochemical Profile and Antioxidant Activity of *Phoenix dactylifera* L., *Phoenix canariensis*L. and *Chamaeropshumilis*L. Acta Hort. 882:1099-1108.

Boulenouar, N., Marouf, A. and Cheriti, A. 2011.Antifungal activity and phytochemical screening of extracts from Phoenix dactylifera L. cultivars. Nat. Prod. Res. 25:1999-2002.

Cavin, A., Hostettmann, K., Dyatmyko, W. and Potterat, O. 1998. Antioxidant and lipophilic constituents of TinosporaCrispa. Planta Med. 64:393-396.

Cuendet, M., Hostettmann, K. and Potterat, O. 1997.Iridoid glucosides with free radical scavenging properties from Fagraeablumei.Helv.Chim.Acta 80:1144-1152.

Eddine, L.S.,Segni, L. and Ridha, O.M. 2014a. Composition, in vitro antioxidant activity, and antimutagenic capacity of leaf extract of *Phoenyxdactylifera*L from Algeria. Journal of Pharmacy Research 8:233-239.

Eddine, L.S., Segni, L., Noureddine, G., Redha, O.M. and Sonia, M. 2014b. Scavenging Effect, Anti-Inflammatory and Diabetes Related Enzyme Inhibition Properties of Leaves Extract from Selected Varieties of *Phoenix dactylifera* L. International Journal of Pharmacognosy and Phytochemical Research 6:66-73.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. 1961.A new and rapid colorimetric determination of acetylcholinesterase activity.Biochem.Pharmacol. 7:88-95.

Haimoud, S.A., Allem, R. and Merouane, A. 2016. Antioxidant and anti-inflammatory properties of widely consumed date palm (Phoenix Dactylifera L.) fruit varieties in algerian oases. J. Food Biochem. 40:463-471.

Ingkaninan, K., De Best, C.M., Van Der Heijden, R., Hofte, A.J., Karabatak, B., Irth, H., Tjaden, U.R., Van Der Greef, J. and Verpoorte, R. 2000. High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products. J. Chromatogr. A 872:61-73.

Mansouri, A., Embarek, G., Kokkalou, E. and Kefalas, P. 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chem.89:411-420.

Pinho, B.R., Ferreres, F., Valentão, P. and Andrade, P.B. 2013. Nature as a source of metabolites with cholinesterase-inhibitory activity: an approach to Alzheimer´s disease treatment. J. Pharm. Pharmacol. 65:1681-1700.

Sekeroglu, N., Senol, F.S., Orhan, I.E., Gulpinar, A.R., Kartal, M. and Sener, B. 2012.*In vitro* prospective effects of various traditional herbal coffees consumed in Anatolia linked to neurodegeneration. Food Res. Int. 45:197-203.

Selmani, C., Chabane, D. and Bouguedoura, N. 2017. Ethnobotanical survey of *Phoenix dactylifera* L. pollen used for the treatment of infertility problems in Algerian Oases. J. Evid. Based Complementary Altern. Med. 14:175-186.

Wagner, H. and Bladt, S. 1996. Plant drug analysis, a thin layer chromatography atlas.Springer, Berlin.

Zohra, R., Redha, O.M. and Eddine, L.S. 2016. Evaluation of Phenolic Content and Antioxidant Capacity of leaf extract from *Phoenix Dactylifera L* obtained by different pH of aqueous extraction. Journal of Pharmacy Research 10:1-7.

Tables

Table 1. Extract yield, antioxidant and acetylcholinesterase inhibitory activities of extracts of *Phoenix dactylifera*.

Extract	Yield (%)	IC_{50}[2,3] DPPH (µg/ml)	IC_{50}[2,3] AChE (µg/ml)
Leaves			
Dichloromethane	6.54	403.91 ± 1.54^{s}	n.a.
Methanol	14.37	13.48 ± 0.18^{t}	382.05 ± 10.68^{s}
Aqueous	8.71	119.47 ± 2.71^{u}	n.a.
Phenolic	1.96	53.08 ± 0.61^{v}	310.58 ± 0.74^{t}
Pollen			
Dichloromethane	2.1	493.92 ± 11.23^{s}	n.a.
Methanol	17.71	1116.83 ± 29.40^{w}	n.a.
Aqueous	25.33	530.99 ± 1.62^{x}	n.a.
Phenolic	2.43	56.22 ± 0.19^{v}	n.a.
Standards			
Ascorbic acid[1]	-	3.06 ± 0.02^{y}	-
Quercetin[1]	-	3.01 ± 0.01^{y}	-
Trolox[1]	-	4.89 ± 0.01^{y}	-
BHT[1]	-	23.67 ± 0.46^{z}	-
Galanthamine[1]	-	-	0.30 ± 0.0021^{u}

[1] Compound used as positive control.
[2] Standard error of the mean of three assays.
[3] Concentration that shows 50% activity.
[s-z] Differences within columns (samples not connected by the same letter are statistically different at $p < 0.05$).
n.a. not active.

Figures

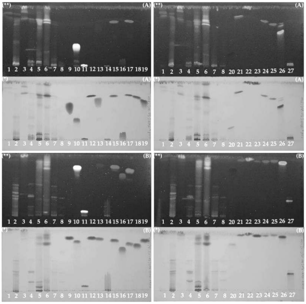

Fig.1.TLC analysis of extracts of *Phoenix dactylifera*. TLC plates were developed by spraying *Natural products-polyethylene glycol reagent*. Mobile phase **(A)**: chloroform : methanol (80 : 20; v/v), **(B)**: ethyl acetate : methanol : water (100 : 13.5 : 10; v/v). Tracks: **1** - dichloromethane extract (leaves), **2** - methanol extract (leaves), **3** - dichloromethane extract (pollen), **4** - methanol extract (pollen), **5** - phenolic extract (leaves), **6** - phenolic extract (pollen), **7** - aqueous extract (leaves), **8** - aqueous extract (pollen), **9** - benzoic acid, **10** - caffeic acid, **11** - chlorogenic acid, **12** - cinnamic acid, **13** - *para*-coumaric acid, **14** - ellagic acid, **15** - ferulic acid, **16** - gallic acid, **17** - sinapic acid, **18** - syringic acid, **19** - vanillic acid, **20** - catechin, **21** - chrysin, **22** - flavan, **23** - flavanone, **24** - hesperetin, **25** - naringenin, **26** - quercetin, **27** - rutin. The applied amounts; plant extracts: 250 µg; standards: 25 µg. **(*)**: 254 nm, **(**)**: 365 nm.

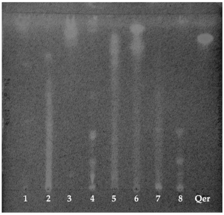

Fig. 2. Bioautography of extracts of *Phoenix dactylifera*. Antioxidant activity was developed by spraying the TLC plates with 0.2% DPPH solution. Mobile phase: ethyl acetate : methanol : water (100 : 13.5 : 10; v/v). Tracks: **1** - dichloromethane extract (leaves), **2** - methanol extract (leaves), **3** - dichloromethane extract (pollen), **4** - methanol extract (pollen), **5** - phenolic extract (leaves), **6** - phenolic extract (pollen), **7** - aqueous extract (leaves), **8** - aqueous extract (pollen), **Qer** - quercetin. The applied amounts; plant extract: 25 µg; standard: 10 µg.

Effectiveness of the arbuscular mycorrhizas in the protection of date palm against dry conditions of arid lands

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Abstract

Date palm (*Phoenix dactylifera* L.) is considered crucial for the oasian ecosystem as it protects the surrounding vegetation against desert influences and provides adequate microclimate to the under storey crops. Although date palm is thought drought tolerant, water is required to fully realize growth and productivity performance, thus its yield and productivity can be negatively affect by water shortage. Date palm adaptation to water scarcity is mostly the result of existing intrinsic traits. However, arbuscular mycorrhizal (AM) symbiosis is widely believed to provide complementary characteristics that improve host plants protection against the deleterious effect of drought. The multiple benefits gained from AM symbiosis can be characterized agronomically by increased growth and yield, physiologically by improved nutrients and water status, and ecologically by improved ecosystem stability. Mycorrhiza performances are more pronounced under harsh conditions including poor soil, water scarcity and soil salinity. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental constraints than are non-mycorrhizal plants. In this presentation, we will discuss our recent results concerning AMF effectiveness in increasing date palm performance in terms of growth, nutrition and protection against the detrimental effect of drought. In short, the contribution of AMF in date palm tolerance to water stress was attributed to enhanced water status and nutrient acquisition under short-term drought, and to enhanced protection against oxidative stress as evidenced by the increased antioxidant enzyme activities and the alleviation of H_2O_2 and MDA accumulation in mycorrhizal plants subjected to long period of drought.

Keywords : Date palm ; Arbuscular mycorrhizal fungi ; drought ; water relation ; Oxidative stress.

INTRODUCTION

Over the history, date palm tree has played a major role in the life of human beings as a major agricultural crop. It could be used for generations to come due to the remarkable nutritional and economic value of its fruits “dates” and its capacity to produce in harsh environment where other fruit trees production is limited. Oasis ecosystem is often subjected to sever environmental constraint such as poor soils and long-term drought causing considerable soci-economic and ecological damages (Haddouch, 1997). Among abiotic stresses, waters scarcity is the most severe environmental stress impairing date palm development in arid and semi-arid areas where long dry seasons with low water availability adversely affects all plant functions. Therefore, there is an urgent need to develop alternatives to alleviate drought-related impacts. In this context, arbuscular mycorrhizal (AM) symbiosis (mycorrhiza) is widely known to provide complementary characteristics that improve host plants protection against the detrimental effects of environmental constraints (Giri et al. 2003; Goicoechea et al. 2004; Faghire et al. 2010; Fouad et al., 2014). Mycorrhiza refers to a highly evolved mutualistic association between plants and soil borne fungi (AM fungi) that colonize the cortical tissue of roots during periods of active plant growth. 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.In arid and semi-arid areas, AMF may represent not only a biological mean to counteract environmental constraints, but also a challenge for the development of the long-term agricultural production. The ability of the root systems to establish beneficial symbiotic relationships with AM fungi (AMF) represents one of the most successful strategies that land plants have developed to cope with abiotic stresses (Giri et al. 2003; Garmendia et al. 2004; Goicoechea et al. 2004; Fouad et al., 2014). The purpose of this paper is to assess the response of mycorrhizal date palm to long period of drought in terms of water status and antioxidant metabolism activation.

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 mycorrhizal (NM) 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. The beneficial effects of AMF was assessed, after 2 and 6 months, by measuring parameters related to plant growth and biomass production (shoot and root dry weights), and plant water status as previously described (Faghire et al., 2010). Leaf water potential (Ψ_w) was measured by the method of chamber pressure developed by Scholander *et al*. (1965). Osmotic potential at full turgor (Ψ_π^{100}), osmotic potential at turgor loss ($\Psi\pi_0$), symplastic water (W_S), and cell elasticity modulus (ξ) were obtained from the pressure-volume curve method (Tyree and Hammel, 1972).

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., (2010). 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. 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 (table 1). Improved growth under short period of water stress was 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 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+. Indeed tissue nutrients (P, K, Ca and Mg) concentrations were higher in mycorrhizal plants (AM) than in non-inoculated (NM) ones. Water stress induced a significant decrease of plant nutrient concentrations (Table 1). This negative effect was more pronounced in NM than in AM plants. These Results were in accord with previous finding in date palm (Oihabi, 1991; Faghire et al. 2010; Baslam et al., 2014; Benhiba et al., 2014; Benhiba et al., 2015) and other plant species (Ruiz-Lozano and Azcon, 1996; Wu and Xia, 2005; Fouad et al., 2014; Essahibi et al., 2017). 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; Fouad et al., 2014). 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.

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 NM 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 NM 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 $\Psi\pi$ 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 have been correlated with drought-adaptation and may provide cells with a high resistance to water stress (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 strengthening plants tolerance to long period of water stress 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; Fouad et al. 2014; Essahibi et al., 2017). 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; Fouad et al. 2014; Essahibi et al., 2017). 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 long period of drought.

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 first instance to differences in tissue hydration between AM-P and N-AM plants. However, AM symbiosis enhance drought tolerance of host plants through many other mechanisms. 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 plants subjected to lont period of water stress, and this was correlated to plant protection against drought.

REFERENCES

Aebi H., 1984. Catalase in vitro. Method Enzymol 105: 121-126.

Amako K., Chen GX. , Asada K., 1994. Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant Cell Physiol 35: 497-504.

Baslam M., Faghire M ; Samri S., Meddich A., Goicoechea N., Qaddoury A., 2010. Effect of arbuscular mycorrhizal fungi on date palm response to water deficit. International Symposium on the Biology of the Palm family, PALM 2010, 5-7 Mai 2010, Montpellier, France.

Baslam M., Qaddoury A, Goicoechea N. 2014. Role of native and exotic mycorrhizal symbiosis to develop morphological, physiological and biochemical responses coping with water drought of date palm, Phoenix dactylifera. Trees, 2014: 161-172

Benhiba L., Essahibi A., Fouad M.O., Qaddoury A. **2014**. Mycorrhizal symbiosis alleviated reactive oxygen species accumulation in date palm under water deficit. Proceedings of the Fifth International Date Palm Conference, pp: 349-354

Benhiba L., Fouad M.O., Essahibi A., Qaddoury A., 2015. Arbuscular mycorrhizal symbiosis enhanced growth and antioxydants metabolism in date palm seedlings subjected to long-term drought. Trees (2015) 29: 1725-1733

Beyer WF, Fridovich I, 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161: 559-566.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72: 248-254.

Bucher, M., 2007, Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol. 173: 11–26.

Abdellatif ESSAHIBI, Laila BENHIBA, Mohamed AIT BABRAM, Cherki GHOULAM and Ahmed QADDOURY. (**2017**). Influence of arbuscular mycorrhizal fungi on the functional mechanisms associated with drought tolerance in carob (*Ceratonia siliqua* L.). *Trees, 32: 87-97.*

Faghire M; Baslam M.; Samri S., Meddich A., Goicoechea N., Qaddoury A., 2010. Effect of arbuscular mycorrhizal colonization on nutrient statut, water relations and growth of date palm seedlings under water stress. Acta Hort. 2010, 882.

Fouad MO, Essahibi A, Benhiba A, Qaddoury A (2014) Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. Span J Agric Res 12:763–771

Garmendia I, Goicoechea N, Aguirreolea J (2004) Antioxidant metabolism in asymptomatic leaves of Verticillium-infected pepper associated with an arbuscular mycorrhizal fungus. J Phytopathol 152:593–599

Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of Acacia auriculiformis. Biol Fertil Soils 38:170–175

Goicoechea N, Merino S, Sa´nchez-Dı´az M (2004) Contribution of arbuscular mycorrhizal fungi (AMF) to the adaptations exhibited by the deciduous shrub Anthyllis cytisoides under water deficit. Physiol Plant 122:453–464

Haddouch M. 1997. Situation actuelle et perspective de développement du palmier dattier au Maroc. Option Mediteranéennes, serie A : séminaires méditerranéennes, n°28. Le palmier dattier dans l'agriculture d'oasis des pays méditerranéennes.

Heat and Pachker., 1981. Biochem. Biophys.Res common 19. 716-720 (1965) J.Exp.Bot. 32 : 93-101

Kehri, H.K., and Chandra, S., 1990, Mycorrhizal association in crops under sewage farming.J. Indian Bot. Soc. 69: 267–270.

Liu, A., Hamel, C., Elmi, A., Costa, C., Ma, B., and Smith, D.L., 2002, Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. Can. J. Soil Sci. 82: 271–278

Nelsen CE., and Safir GR., 1982. Planta. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154(5): 407-13

Oihabi A., 1991. Etude de l'influence champignons MVA sur le Bayoud et la nutrition du palmier dattier. Thèse d'état, Univ. Cadi Ayyad, Fac. des sciences, Marrakech, Maroc.

Paquin R, Lechasseur P (1979) Observations sur une me´thode de dosage de la proline libre dans les extraits des plantes. Can J Bot 57:1851–1854

Patterson BD, MacRae EA, Ferguson IB .1984. Estimation of hydrogen peroxide in plant extracts using tetanium (IV) Anal. Biochem 139 : 487-492

Polle A, Otter T, Seifert F, 1994. Apoplastic peroxidases and lignification in needles of Norway (*Picea abies* L.). Plant Physiol 106: 53-60.

Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55:1743–1750

Rhodes, L.H. and Gerdemann 1980. Nutrient translocations in VA mycorrhizae. pp 173 ff in: C.W. Pappas and E.D. Rudolph (ed): Cellular interactions in symbiosis and parasitim. The Ohio State University Press, Colombus, Ohio.

Ruiz-Lozano J.M. ; Azcon R., 1996. Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants, Agric. Ecosyst. Environ. 60, 175–181.

Scholander PF, Hammel HT, Hemmingsen EA. 1965. Sap pressure in vascular plant. Science 148:339–346

Tyree, M.T. and H.T. Hammel., 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J. Expt. Bot. 23:267-282.

Wu Q.S. and Xia R.X., 2005. Arbuscular mycorrizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Plant Physiology 163: 417-425.

Yemm EN, Willis AJ., 1954. The estimation of carbohydrates in plants extracts by anthrone. Biochem J 57:508–514

Tables

Table 1: Mycorrhizal colonization, Plant height, Root length, shoot dry weight and root dry weight and nutrients contents in non-mycorrhizal (NM) or mycorrhizal (AM) date palm seedlings grown under well watered (WW) or water stress (WS) conditions for 8 weeks .

Water status	AMF status	M (%)	SDW (g)	RDW (g)	SH (cm)	RL (cm)	P (mg)	Ca (mg)	Mg (mg)	K (mg)
WW	NM	0	3.5b	1.62b	28.3c	35.3c	1.7b	0.5b	0.7b	1.4c
	AM	53.1a	4.9a	2.68a	31.8a	31.9a	6.1a	0.9a	1.94a	2.4a
WS	NM	0	1.37c	0.63c	23.3d	23.3d	1.6b	0.23c	0.4b	0.7d
	AM	46.2b	2.81b	1.14a	29.3b	29.4b	5.8a	0.6b	2.2a	2b

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2: Relative water content (RWC), turgid weight dry weight ratio (TW/DW), Leaf water potential (Ψ_w), Osmotic potential at full turgor (Ψ_π^{100}), Osmotic potential at turgor loss ($\Psi\pi_0$), cell Modulus of Elasticity (ξ), and Symplastic water (W_S) in mycorrhizal (AM) and non-mycorrhizal (NM) date palm seedlings subjected to well water (WW) or water stress treatment for 8 weeks .

Water status	AM statut	RWC (%)	TW/DW	Ψ_w (-Mpa)	Ψ_π^{100} (-Mpa)	Ψ_π^0 (-Mpa)	ε	SW
WW	AM	99.1 a	1.9b	27.2cd	6.9c	16.6c	3.5 a	5.5a
	NM	98.6 a	2.4a	30.5b	4.8c	15.3c	1.8 b	5.6a
WS	AM	96.9ab	1.6c	28c	13.3b	25b	0.34c	3.8c
	NM	92.4 b	2.1ab	33.5a	21a	28.5a	1.3b	2.5b

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 (OD) in leaves of non-mycorrhizal (NM) or mycorrhizal (AM) date palm seedlings grown under well water (WW) or water stress (WS) conditions for 6 months.

Water status	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	NM	53.2b	24.1b	54.6c	4105.2c	12.42b
	AM	38.6d	24.2b	78.7a	9193.2a	6.88c
WS	NM	65.5a	28.4a	47.5d	3570.2d	20.47a
	AM	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 (NM) or mycorrhizal (AM) date palm seedlings grown under well water (WW) or water stress (WS) conditions for 6 months.

Water status	AMF status	SOD (USOD g^{-1} DM min^{-1})	G-POD (µmol.g^{-1} DM min^{-1})	CAT (nmol g^{-1} DM min^{-1})	APX (µmol g^{-1} DM min^{-1})
WW	NM	652,3a	3,07c	116,7cd	2,11d
	AM	447,7c	4,20b	136,8c	2,89d
WS	NM	596,1b	4,44b	225,5a	4,68c
	AM	667,4a	5,87a	104,41d	7,05a

Values within each column followed by the same letter are not significantly different ($p \leq 0.05$).

Comparative performance of date palm varieties for production of fresh and dry dates under green glass house conditions

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Abstract

Traditional methods of open sun drying were initially used for Post-Harvest Processing of dates, later on the practice of covering fruit bunches with bitumen paper to protect it from monsoon rains were started, but, the desired quality of fruit could not be achieved. Due to adverse effects of climate change i.e. dust storms and rain spells; occurring especially at the stage of ripening and curing of fruits, the farming community switched to solar tunnel drying. Even though, their expectations could not be achieved. Temperature and humidity are the two main parameters which affect the date fruit quality. At ripening stage the fruit needs low temperature and high humidity, while; during drying it requires high temperature and low humidity. Controlled ripening, as compared to natural ripening, may be the best option to overcome the problems caused by long time exposure to climatic atrocities; while staying on the palm trees. In controlled ripening the dates may be converted from "Khalal" to "Tamar" stage which reduces the chance of detachment of the outer skin fruit from its flesh. This research study was carried out at Arid Zone Research Center, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan for early and safe ripening of dates under controlled temperature and humidity levels on most popular varieties of date palm in the area. For ripening of the dates $\pm 35^0$C temperature & $\pm$80% relative humidity and for drying $\pm 45^0$C temperature and $\pm$30% relative humidity levels were kept as constant factors. The moisture contents were reduced to the recommended water activity level of 23-25% for shelf life stability in all varieties under study. Data depicted that fresh date produce ranged from 39% to 59%; however minimum (39%) fresh date were recorded in cv. Gulistan and maximum produce was recorded in fresh dates of Shakri variety (59%). Similarly, the minimum and maximum produce recorded in dry dates was 32% (Basara variety) and 50% in Zahidi. The maximum and minimum size observed was 5.2cm and 2.5cm in case of Dhakki and Basra varieties respectively. As an overview of the study it can be recommended that farmer can fetch more from their produce if they process fresh dates rather than dry dates in case of most of the popular varieties.

Keywords: Monsoon, Tamar, Khalal, Dates, Postharvest, Ripening, Green Glass House, Water Activity Level, Physical Properties.

INTRODUCION

The date palm (*Phoenix dactylifera* L.) is considered as one of the most important fruits of Pakistan. More than 150 varieties of dates are grown in Pakistan which includes Dhakki, Shakri, Zahidi, Baitullah, Muzalti, Gulistan, Basra etc (Nadeem et al., 2011). Many of these dates are unique in taste, texture and response of commune's acceptability.

Dates are mainly produced in arid regions having high temperatures, low humidity and abundant water for growth. Baluchistan and Sindh are the two major provinces contributing around 85% to 90% to the total date production of Pakistan. Some parts of

Punjab and Khyber Pakhtunkhwa also produce a few varieties and add up to the national total (SBI 2010).

The maturity stages of dates are characterized as "Khalal or Kimri", "Rutab" and "Tamar". Moisture contents of dates at "Khalal" stage range from 45 to 65%, at "Rutab" stage the moisture contents are 30-45% and at "Tamar" stage the moisture content is less than 30%. At "Tamar" stage the sugar content is about 60 to 80% (Al-Hooti et al., 1997). At this stage the product is suitable for long time storage and improved shelf life. Most dates are harvested at "Rutab" stage. At this stage the dates are fully-ripen soft and light brown in color. At "Tamar" stage the dates are dark brown in color, soft and semidry or dry (Farah et al., 2013).

Dates have a valuable contribution in the farmers' income. A number of physical changes and problems in the final harvested fruits are caused by natural ripening of date fruits on palm trees. It includes blistering (detachment of the date flesh from pit), sticking of unwanted impurities to the fruits and increasing its microbial load (Sakr et al., 2010). Due to these factors the dates do not reach to the "Rutab" stage and causes loss in huge quantities. Technological processes such as washing, cleaning and packing are carried for further value addition and proper post harvest management of dates. Under such circumstances the dates are not available in fresh form and are converted into dry dates. There are three factors due to which the fresh dates are converted into dry dates.

- Due to climate change, there is one big threat in the form of monsoon season which coincides with the harvesting season of dates every year. It adversely affects its availability in fresh form.
- Inida is the world's largest market for dry dates which add up to the economy of dates producing countries.
- Unavailability of appropriate cold storages and processing facilities.

Controlled ripening, as compared to natural ripening, may be the best option to overcome the problems caused by long time exposure to climatic atrocities; while staying on the palm trees. In controlled ripening the dates may be converted from "Khalal" to "Tamar" stage which reduces the chance of detachment of the outer skin from the inner one (Navarro, 2006).

Traditional methods of *open sun drying* were initially used for Post-Harvest Processing of dates. Later on the practice of *covering fruit bunches with bitumen paper* to protect it from monsoon rains was adopted, but, the desired quality and quantity of fruit could not be achieved. Due to adverse effects of climate change, resulting in dust storms and rain spells especially at the stage of ripening and curing of fruits, the farming community switched to *dry date processing* (Ref)

Temperature and *humidity* are the two main contributors affecting the fruit quality. At ripening stage the fruit needs low temperature and high humidity, while; during drying it requires high temperature and low humidity. In all the previous research work carried out on solar tunnel drying, only one parameter i.e. temperature was considered (SBI 2010). At ripening stage, the temperature inside the dryer was raised much enough and humidity was not taken in consideration, due to which the flesh got detached from the pit and the desired quality products were not achieved. Moreover in case of world fame Jambo size date palm cv. Dhakki could not mature on plant and consequently its ripening is done at ground in open sun shine.

In the Glass House at Arid Zone Research Center, Dera Ismail Khan, eight different varieties of dates were processed for comparing their economic return in form of fresh dates as compared to dry dates of the same varieties. These varieties included Shakri, Zahidi, Baitullah, Muzalti, Gulistan, Shakri Yellow, Basra and Dhakki.

MATERIAL AND METHODS

The study was carried out at Arid Zone Research Center, Dera Ismail Khan in August 2017.

Harvesting of Dates:

Date varieties including Shakri, Zahidi, Baitullah, Muzavti, Gulistan, Shakri Yellow, Basra and Dhakki were harvested from selected plant of the orchard at Arid Zone Research Center, Dera Ismail Khan at Khalal stage; meant for experimentation.

Pre-treatment:

Harvested dates were collected in bunches, detached from bunches; through adverse side pushing for ensuring the caps attachment. The fruits were washed with clean water to remove dust and other inert material. Sorting was carried out to remove damaged and spoiled fruit (El Mardi et al., 2006).

Sample size:

One kilogram sample of each variety was collected from the sorted fruits and replicated three times for processing both fresh date and dry dates. The samples collected for dry dates were immersed in boiling water for five minutes and were placed in perforated trays for processing. While samples meant for fresh date processing were placed on perforated trays after washing with clean water (Ahmad et al., 1995). Both samples were shifted to Green Glass House.

Glass House Operation:

The components of Glass House installed at Arid Zone Research Center, D.I Khan include ventilators, exhaust fans, blowers, cooling pad, misters and heating cables. For rising inside temperature, the entrance and ventilators were closed and all the running components were shut down. Similarly, for lowering inside temperature, the entrance and ventilators were opened and the cooling pad, exhaust fans and blowers were switched on to blow out the trapped hot air. For increasing the inside humidity, entrance and ventilators were closed while the cooling pad and misters were switched on. In short the humidity and temperature were controlled and kept at constant levels i.e. for ripening of the dates $\pm 35^0$C temperature & $\pm$80% relative humidity and for drying $\pm 45^0$C temperature and $\pm$30% relative humidity (Farah et al., 2013).

Drying and Dehydration:

To preserve the quality and for safe storage the moisture contents of date fruit were reduced to desired moisture content (23-25%). To keep the dates soft the moisture content less than 20% should be avoided (Kader, 2009).

Physical Properties of Dates:

At Khalal stage, the shape, color and size of all collected samples were observed and presented in tabular form (Table 1).

On the basis of length and diameter, date fruits were categorized as small, medium and large sizes; as compared to each other. The ranges of fruit size were set as small fruit <4.0cm, for medium fruit 4.0–5.0cm and large fruit >5.0cm (Markhand et al., 2010).

Data recording:

Data including temperature and humidity inside GGH and texture of dates was recorded on daily basis. Both dry dates and fresh dates were re-weighed when attained 0.52 water activity levels (Ahmad et al., 2007) and attaining full ripening respectively.

RESULTS AND DISCUSSION

Eight different varieties of dates were processed in Green Glass House under controlled conditions. The average temperature maintained in GGH was 45ºC and the average humidity was 75% and the response of different varieties of dates was as follows.

The average weight loss as compared to initial samples; in "Shakri" was 41% for fresh dates, while; it was 56% for producing dry dates. The average weight loss in "Zahidi" was 50% for fresh dates, while; it was 50% for producing dry dates. The average weight loss in "Baitullah" was 51% in case of fresh dates, while; it was 60% for producing dry dates. The average weight loss in "Muzawti" was 51% in case of fresh dates, while; it was 52% for producing dry dates. The average weight loss in "Gulistan" was 61% in case of fresh dates, while; it was 52% for producing dry dates. The average weight loss in "Shakri Yellow" was 52% in case of fresh dates, while; it was 60% for producing dry dates. The average weight loss in "Basra" was 57% in case of fresh dates, while; it was 68% for producing dry dates. The average weight loss in "Dhakki" was 50% in case of fresh dates, while; it was 64% for producing dry dates. The average minimum weight loss of 41% occurred in fresh dates produced from Shakri variety and average maximum weight loss of 61% occurred in fresh dates produced from Gulistan variety. Similarly, the average minimum weight loss of 50% and average maximum weight loss of 68% occurred in dry dates produced from Shakri variety.

Dry dates produced from "Baitullah" variety were of good quality, but, fresh dates of good quality could not be produced from this variety. The fresh dates of "Baitullah" variety became bone dry, its flesh got detached from the pit and its skin got ruptured. "Dhakki" variety on the other hand produced both good quality fresh as well as dry dates. The fresh dates produced were good in color and taste. The produce was also fleshy and large in size as compared to the other seven varieties.

The maximum and minimum size observed among varieties under study was 5.2cm and 2.5cm in case of Dhakki and Basra varieties respectively. It is depicted from table 3 that fresh date market rates of almost all varieties is greater than rates of dry date except Gulistan wherein due to comparatively high water activity level fresh form is very perishable. To reduce the moisture content for safe storage this variety is converted into dry dates and, hence, is not available in fresh form. Moreover, it is more fleshy and soft to maintain its shape during processing and packaging. Though the fresh dates production from unit mass of Dhoka was varying from 39% to 59%, however, in Toto it was observed that dates processing for fresh dates production was more economical and worth paying to the growers than processing for dry dates. From Table 4 it can be concluded that fresh dates gave double return as compared to dry dates.

RECOMMENDATIONS

Fresh dates of "Baitullah" variety were of inferior quality. Thus only dry dates can be prepared from this variety. The other option is to process this variety under lower temperature and high humidity ranges to avoid maximum moisture loss.

REFERENCES

Nadeem M, Salim-ur-Rehman, Anjum FM and Bhatti IA. 2011. Quality evaluation of some Pakistani date varieties. Pak J Agric Sci 48(4): 305-313.

Shamim F, Muhammad AA, Muhammad A, Ahmad D, Irum B and Zarina Y. 2013. Controlled ripening of date palm fruit and impact on quality during post harvest storage. Ext J App Sci 1(2): 53-57.

Sakr MM, Abu-Zeid IM, Hassan AE, Baz AGIO and Hassan WM. 2010. Identification of some Date palm (*Phoenix dactylifera*) cultivars by fruit characters. Indian J Sci and Technol 3(3): 338-343.

Navarro S. 2006. Postharvest treatment of dates. Stewart Postharvest Review. 2(1): 1-9.

Sindh Board of Investment. 2010. Dates Drying and Dehydration Plant.

Baloch, A.K., Baloch, W.A., Baloch, M.K. and Saleem, S.A. 2007. Shelf Stability of Dhakki Dates As Influenced By Water Activity And Headspace Atmosphere. Acta Hort. (ISHS) 736:575-586.

Kader, A.A. and Hussein, Awad. 2009. Harvesting and postharvest handling of dates. ICARDA, Aleppo, Syria. iv + 15 pp.

Markhand, G.S., Adeel, A.A., Ameer, A.M. and Nisar, A.K. 2010. Fruit Characterization of Pakistani Dates. Pak. J. Bot. 42(6): 3715-3722.

Abdessalam, M., F. C. Ali, C. Nizar, S M. Ben, B. Mohammad and M. P. Threadgill. 2008. Physio-chemical characteristics and total quality of date palm varieties grown in the southern of Tunisia. Pak. J. Biol. Sci. 11 (7):1003-1008.

Ahmad, I.A., A.W.K. Ahmad and R.K. Robinson. 1995. Chemical composition of date varieties as influenced by stage of ripening. Food Chemistry. 46(2): 305-309.

Al-Hooti, S., J. S. Sidhu and H. Qabazard. 1997. Physico-chemical characteristics of five date fruit cultivars grown in the United Arab Emirates. Plant Foods for Human Nutrition, 50(2): 101-113.

El Mardi, M.O., F.A.J. Al-Said, C.B. Sakit, L.M. Al-Kharusi, I.N. Al Rahbi and K. Al Mahrazi. 2006. Effect of pollination method and fertilizer and mulch treatments on the physical and chemical characteristics of date palm (*Phoenix dactylifera*) fruit: Physical characteristics. Third Int. Date Palm Conf. Abu Dhabi, Feb. 19-21.

Tables

Table 1: Physical Properties of Different Varieties of Dates

S/No.	Variety	Color	Shape	Length (cm)	Size
1	Shakri	Medium Brown	Oval	3	Small
2	Zahidi	Golden Yellow	Oval	3.5	Small
3	Baitullah	Golden Brown	Cylindrical	3	Small
4	Muzawti	Black	Thick Oval	2.5-4.5	Small to Medium
5	Gulistan	Light Brown	Oval	4.5	Medium
6	Shakri Yellow	Yellowish Brown	Oval	3	Small
7	Basra	Yellow	Oval	2.5	Small
8	Dhakki	Light Brown	Oblong Oval	5.2	Large

Table 2: Weight Loss of Different Varieties of Dates

S/No.	Variety	Fresh Dates Weight (Kg)			Weight Loss (%)	Dry Dates Weight (Kg)			Weight Loss (%)
		Initial	Final	Difference		Initial	Final	Difference	
1	Shakri	1	0.59 a	0.41 f	41 f	1	0.44 c	0.56 d	56 d
2	Zahidi	1	0.50 b	0.50 e	50 e	1	0.50 a	0.50 f	50 f
3	Baitullah	1	0.49 c	0.51 d	51 d	1	0.40 d	0.60 c	60 c
4	Muzawati	1	0.49 c	0.51 d	51 d	1	0.48 b	0.52 e	52 e
5	Gulistan	1	0.39 f	0.61 a	61 a	1	0.48 b	0.52 e	52 e
6	Shakri Yellow	1	0.48 d	0.52 c	52 c	1	0.40 d	0.60 c	60 c
7	Basra	1	0.43 e	0.57 b	57 b	1	0.32 f	0.68 a	68 a
8	Dhakki	1	0.50 b	0.50 e	50 e	1	0.36 e	0.64 b	64 b

Table 3: Comparative Economics

Variety	Fresh Dates			Dry Dates			Fresh Dates	Dry Dates
	Weight Obtained after processing (Kg)	Market Rate (Rs)	Cost (Rs)	Weight Obtained after processing (%age of the initial sample)	Market Rate (Rs)	Cost (Rs)	Comparatively More Money Return (%)	
Shakri	59%	100	59	44%	120	53	10	
Zahidi	50%	150	75	50%	90	45	40	
Baitullah	49%	100	49	40%	85	34	31	
Muzafati	49%	120	59	48%	100	48	19	
Gulistan	39%	100	39	48%	150	72	Fresh date preparation not possible	
Shakri Yellow	48%	120	58	40%	100	40	31	
Gol Basra	43%	75	32	32%	100	32	Equal return	
Dhakki	50%	300	150	36%	250	90	40	

Table 4: Comparative Economic Analysis of Fresh & Dry Dates (Chuhara).

Description	Cost (PKR)/Kg	Benefit (PKR)/Kg	BCR
Fresh Dates	70	200	2.8
Dry Dates	118	125	1.1

Integrated Pest Management for control the green date palm pit scale insect (*Palmapsis phoenicis Rao.*) (Homoptera: Asterolecaniidae) in Sudan

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Abstract

Date palm *(Phoenis dactylifera* L.) in Sudan is an economic and food security crop. Estimated annual date production from 8 million date palm trees is about 431.000 mt which is far below the country's potential. Sudan has been famous in the world for production of dry dates. Six good local commercial cultivars are available and research is coming up with better composition of cultivars by local selection and foreign introduction from tissue laboratories.

Sudan is still free from-devastating red palm weevil and the destructive bayoud caused by *Fusarium oxysporum* f.sp. *albedensis.* The green date palm scale insect as an exotic pest appeared in Golid area (1974), Considered in the other palm groves of the world to be of minor or no importance, developed here in a very explosive way. Total number of infested trees is 1200,000. In the past, and due to lack of indigenous knowledge of appropriate control measures adopted to control the date palm green pit scale insect, in Sudan, the treatment control efforts using foliar application of contact insecticides and Mineral oils were not successful (91-1992). Hence the level of infestation steadily increased. Following intensive research an Integrated Pest Management (IPM) was starting with cultural practices or sanitary measures, supplemented with chemical control using systemic insecticides of Neonicotinoid (thiamethoxam & imidacloprid) such as Confidor 200 SL and Actara 25 WG and impact of natural enemies together with plant quarantine legislations. A comprehensive program by Plant Protection Directorate (PPD) has been conducted in infested areas. Sustainable biological control of the green pit scale is very important. Results of surveys revealed that many natural enemies associated with insect have been recorded, the nitidulid beetle Predators found in all surveyed areas were Nitidulidae beetle *Cypocephalus dudichi* L., *lady bird Pharoscymnus numidicus , Chrysoperla sp and the* parasitoid *Metaphycus sp. was found in* association with green pit scale insect and the percentage of parasitism was 16% in some areas.

Key words: Date palm, green pit scale insect, systemic insecticides, Biological control

INTRODUCTION

Sudan is a vast country with an area of 1.8 million km^2. It lies between latitudes 10^o and 22^o N and longitudes 22^o to 38^o E. Its landscape consists primarily of gently sloping plain, with the exception of Jebel Marra Masssif, Red Sea Hills and Nuba Mountains. Mean annual temperatures vary between 26^{o}C and 32^{o}C across the country. The northern part is almost desert and semi desert with average annual temperatures around 30^{o}C and average annual rainfall of about 150 mm. The central part is semi-desert to savannah with average annual temperatures around 27^o C, and average annual rainfall of about 200 mm.

Date palm (*Phoenix dactlylifera*) is believed to be cultivated in northern Sudan and upper Nubia since 3200 BC. Dry date cultivars might have originated in Southern Egypt and Northern Sudan. Date palm *(Phoenis dactylifera* L.) in Sudan is an economic and food security crop. Estimated annual date production from 8 million date palm trees is about

431.000 mt (FAO, 2010), which is far below the country's potential. Sudan has been famous in the world for production of dry dates. Different local and old cultivars and seedling races are known in the country. Six good local commercial cultivars are available and research is coming up with better composition of cultivars by local selection and foreign introduction from tissue laboratories (Khairi, 2015).

However, the date palm industry is facing many serious problems, related to low yields, lack of appropriate packing and presentation as well as limited processing of date products. The low yields in most countries, including Sudan, are due to soil salinity, poor fertility, insect pests and diseases, lack of maintenance and care due to increasing cost of labour and to shortage of personnel trained in improved cultural practices. As a result of the high cost of production and low prices of the produce, farmers tend to neglect or even abandon their orchards. Although the commonly known, insect pests like devastating red weevil and the destructive diseases like bayoud caused by *Fusarium oxysporum* f.sp. *albedensis* ,have not been reported, in Sudan (Ahmed 2013 , Felaero, 2017and Elhassan 2006), the date palm is affected by many biotic factors among which insects are the most important.

In the Sudan, the green date palm pit scale insect**,** *Palmapsis phoenicis* Bodenheimer (*Asterolecanium phoenicis* Bodenheimer) is considered the key pest. This genus, a native of central Asia (Iran), was not known in Sudan before 1989 when it was firstly reported by Ali (1989) in El Golid area, as a result of an introduction of some offshoots from Saudi Arabia in 1974. Later, the pest crossed the natural barrier of Baja desert to invade Elgaba scheme, (150 km south of Dongola, 400km-north of Khartoum) and has become a real threat to date palm cultivation in Northern Sudan. The infested area in El Golid, Elgaba and Old Dongola is about 5000 hectares, extending over 60 and 50 kilometers along the west and east banks of the river Nile respectively. the pest was continued her spread and the infestation extend to Burgag scheme north Dongola, Abuhamad in the River Nile State and Khartoum State with Total number of infested trees is 1200. 000

The insect attacks the leaflets, leaf rachis and fruits. It causes chlorosis, degeneration of the leaves, malformation of fruits before maturity (plate 1) leading to losses in production from a range of 30-50 kg to 5 kg per tree (Ali *et.al.*, 1992, Ahmed 2008). The losses may range between 85 and 90% according to infestation rate, variety infested and management conditions (Ahmed, 2002and 2004). In the past, and due to lack of indigenous knowledge of the nature of this pest, control efforts were not successful; hence the level of infestation steadily increased. Studies have been conducted on the biology and population development of the pest, its seasonal abundance, susceptibility of date palm varieties, losses caused by the pest, and on control methods (Ali, 1989 and Ahmed 2000 &2002). An eradication program was attempted based on pruning, local quarantine and aerial insecticide application. Insecticide applied, at spray volumes of 100 liters of water, were diazinone 60 EC (340 ml), Roger 32% EC (225 ml), and Folimate 80% (200 ml); each including 2 liters of 80% Albolinium oil (Ali *et.al.*, 1992). A 96.4% control rate was achieved, thus lowering the infestation within the targeted area drastically to 3.6% and ended nearly in curtailing apparently its infiltration outside the infested area. The infestation had flared back to more than 50 % in less than one year. Eradication of the insect was doomed to failure. Attempts to locate a biological control agent failed when a Coccinelid beetle was introduced (Ali, 1989). This situation led to the gradual development of an Integrated Pest Management (IPM) program, which was implemented.

Following intensive research an Integrated Pest Management (IPM) was starting with cultural practices or sanitary measures, supplemented with chemical control using systemic insecticides and impact of natural enemies together with plant quarantine legislations. A comprehensive program by Plant Protection Directorate (PPD) has been conducted in infested areas. Sustainable biological control of the green pit scale was implemented based on results of surveys revealed that many natural enemies associated with insect have been recorded, the nitidulid beetle Predators found in all surveyed areas were Nitidulidae beetle *Cypocephalus dudichi* L., *lady bird Pharoscymnus numidicus , Chrysoperla sp and the* parasitoid *Metaphycus sp. was found in* association with green pit scale insect. This paper overviewed the integrated pest management IPM used for control this important pest.

MATERIALS AND METHODS

1. The date palm green pit scale insect-IPM

The IPM approach resulted in successful control of the scale insects, up to the present, whereas agro technical and crop management procedures, including ; cultural practices or sanitary measures, supplemented with chemical control using systemic insecticides of Neonicotinoid (thiamethoxam & imidacloprid) such as Confidor 200 SL and Actara 25 WG and impact of natural enemies together with plant quarantine legislations, were successfully applied to achieve efficient control of the green scale insect (Ahmed 2002,2004and 2008). The overall change in pest management also significantly improved the preservation of natural enemies of the pests in the plantations (Ahmed 2008, Dafalla and Hassan 2010).

1.1. Cultural practices followed as first IPM component:

The following cultural practices were usually carried out (Ahmed, 2002):

a. Pruning, removal of dead leaves and the lowest row of the highly infested leaves.
b. Raising earth around the palm to facilitate irrigation (every tree is irrigated individually)
C. Pre-watering (24 hours) before application (of the insecticides).

1.2. Chemical control

A series of small scale experiments were carried out at Elgaba scheme ,Dabba area and El Golid area during the seasons (2003,2007,2013 and 2017) to evaluate the efficacy of systemic insecticides (belonging to neonictoniods) against the green date palm pit scale insect using two application techniques(soil application and trunk injection).
The systemic insecticide used for this purpose was (imidacloprid as Confidor 200SL or its generic insecticides and thiamethoxam as Actara 75WG) (Ahmed *et .al*, 2002 and 2004).

1.2.1. Soil application method

A. Experimental site:

The experimental sites selected were in Debba area (Elgaba , Elkarad schemes). The trials was conducted during the period of first week of May to the last week of October. The locations selected were two farms, in the middle of the scheme, both locations (location 1) and (location 2) were highly infested, their history of infestation dated about 6 years earlier and the age of trees was between 7 and 10 years with heights ranging between 8 and 10 meters. Intercropping with fodder crops was dominant. Urea and cattle manure were applied to fodder crops. Flood method of irrigation from the Nile is conducted during the flood season. Supplementary irrigation is given using diesel pump from underground water.

Barakawi variety, the most predominant one was selected. A Completely andomized Design with 6 replicates (one tree = replicate) was used imidacloprid formulations (as Confidor 200SL and many generics) 35 ml product /tree (7.0g a.i) and Actara 25 WG (thiamethoxam) 18 ml product /tree (4.5 g a.i) were used beside Untreated control.

The specific dose was diluted with 10 liter of water in a container and drenched around the date palm tree and then irrigated. The irrigation was scheduled at biweekly intervals.

B. Insects count

Samples of eight leaflets (two leaflets from each of the four main directions) were inspected at biweekly intervals and examined under a binocular microscope. The number of living and dead adult females and immature stages were recorded per three cm^2 of each leaflet (tip, top and bottom). An average per cm^2 was obtained and the following parameters were calculated:

a) Mortality of adult females and immature stages of the insect.
b) Percentage mortality of adult females.
c) Percentage mortality of immature stages.

Pre-spray count was undertaken before insecticide application.

C. Yield and yield components

At harvest, triplicate sample of 50 date fruits were taken at random from each replicate, collected samples were used to assess the percentage fruit maturity (ripening). Sub-samples of ten date fruits were taken to the lab to determine the following parameters:

a) Mean fruit weight (g)
b) Mean fruit length(L) (cm)

Yield in kilogram per palm was determined at harvest. Samples of date fruits, soil and grasses were taken to ARC laboratory at Wad Medani for residue analysis.

1.2.2. Trunk injection method

Holes of 15 cm deep were bored into the trunk and an open end snout metallic tube was inserted. The tube; 25 cm in length and 1.5 cm in diameter were inserted into the hole at an angle of 45° about one meter above the ground. The tube can hold at least 25 ml of the diluted insecticide **(**Al-Jbooryi *et.al*, 2001). A developed calibrated "drench mastic" injection gun (used by Fernandez and Gllego, 1997 and Filer, 1973) was not available, so a 25 ml measuring cylinder was used for this purpose. When the injection was over, the tube was closed with tight- fitting flap. Apart from gloves the user also wore a mask for face and eye safety.

The following insecticides were used at the following doses. The injection volume was made upto 25ml using tap-water

Elgaba scheme, season 2003/2004

1) Thiamethoxam as Actara 25 WG at 10 g / tree (2.5 g a.i)
2) Thiamethoxam as Actara 25 WG at 8 g / tree (2.0 g a.i.)
3) Thiamethoxam as Actara 25 WG at 6 g / tree (1.25 g a.i)
4) Imidacloprid as Confidor 200SL at 20 ml / tree (4g a.i.)
5) Imidacloprid as Confidor 200SL at 15 ml / tree (3g a.i.)
6) Imidacloprid Confidor 200SL at 10 ml / tree (2g a.i.)
7) Untreated control (by injecting with water only)

El Golid area, season 2004/2005

1) Thiamethoxam as Actara 25 WG at 2.5 g a.i / tree (10 g product)
2) Thiamethoxam as Actara 25 WG at 2.0 g a.i. / tree (8 g product)
3) Thiamethoxam as Actara 25 WG at 1.25 g a.i. / tree (6 g product)
4) Imidacloprid Confidor 200SL at 4g a.i / tree (20 ml product)
5) Imidacloprid Confidor 200SL at 3g a.i. / tree (15 ml product)

6) Imidacloprid Confidor 200SL at 2g a.i. / tree (10 ml product)
7) Imidacloprid Rinfidor 20%SL at 7g.a. i. / tree (35ml product)
8) Imidacloprid Rinfidor 20%SL at 5g.a.i. / tree (25ml product)
9) Imidacloprid Rinfidor 20%SL at 4g.a.i. / tree (20ml product)
10) Imidacloprid Comodor 20%SL at 7g.a. i. / tree (35ml product)
11) Imidacloprid Comodor 20%SL at 5g.a.i / tree (25ml product)
12) Imidacloprid Comodor 20%SL at 4g.a.i / tree (20ml product)
13) Untreated control (by injecting with water only)

Insects count, yield and yield components and samples for residual analysis as described above.

2. Varietal susceptibility

A study has been conducted by Ahmed (2000 and 2008) in Elgaba scheme, Northern State, Sudan to evaluate the susceptibility of four date palm varieties (Barakawi, Gondiella, Moshrig /Wadlagai and Jaw) to green pit scale insects. About four locations in the infested area were selected (Elgaba scheme ,El Golid area ,Elbaja area and Old Dongola).The four date palm varieties; Barakawi, Gondiella, Waddlagi and Jaw, in the areas were selected and visited monthly for five

months, during the period from August to December 2006. Leaves samples were taken as mentioned earlier. All developmental stages (alive or dead) of the green pit scale insect were counted per leave.

3. Natural enemies' associated with the green pit scale insect in the Northern State, Sudan.

Four locations in the infested area were selected (Elgaba scheme, El Golid area, Elbaja area and Old Dongola). The four date palm varieties; Barakawi, Gondiella, Waddlagi and Jaw, were observed for the natural enemies especially the predators. Ten trees were chosen at random from each variety grown in farms with intercropping or without intercropping. Samples were taken monthly for five months, during the period from August to December 2006. All natural enemies (adults or larvae) were counted per leaf and all developmental stages (alive or dead) of the green pit scale insect were also counted

Monthly counts were conducted over a period of five months (April – December), 2006, covering the four mentioned varieties in the four infested locations.

RESULTS AND DISCUSSION

1. Chemical control

Data obtained from different studies (Ahmed et.al 2005 , Ahmed et. al 2013and Sanhuri et.al (2017) showed the effect of imidacloprid and Actara on Decreasing infestation level and on the average yield per tree. The percentage of ripe fruits (% maturity) and fruit weight (g) and fruit length, L (cm) as physical characteristics. Date palms treated with higher doses of Brodor (35ml), Imidor (35ml), Confidor (35ml) and Actara (18g) showed a high percentage of ripe fruits with no losses. They also showed the highest fruit weight (g) resulting in a higher yield compared to the untreated control.

Partial budget analysis indicated the profitability of the packages recommended by ARC, using systemic insecticides belonging to Neonicotinoid (imidacloprid or thiamethoxam) as indicated by the marginal rate of returns of 364 % for imidacloprid ; Ahmed (2008).

1.1. Soil application method

The results of soil application experiments against the pit scale insect proved that both tested imidacloprid and thiamethoxam compounds reduced the number of the scale pest and they were significantly different from the control (untreated) throughout the test period (12 weeks after application). The higher doses of thiamethoxam as Actara 18 g product /palm and imidacloprid as Rinfidor 200SL and Comodor 200SL (35 ml product/palm) were similar in efficacy to standard insecticide imidacloprid as Confidor 200SL.

Results of this study (Ahmed 2008), conformed the finding of Ahmed (2003) when he evaluate the efficacy of Confidor 200SL and Furadan against the same pest in Elgaba scheme during (1999-2001). Results indicated that dead insect, %mortality (adults and immature stages) were significantly higher than in untreated control after two weeks. All insecticides lost their effectiveness after 12 weeks. The insecticides effectiveness were Confidor (35ml/palm), Furadan 5G (60g/palm) and Confidor (25ml/palm).

Date palms treated with higher doses imidacloprid (35ml) and thiamethoxam (18g) showed a high percentage of ripe fruits with no losses. They also showed the highest fruit weight (g) and the lowest percentage of seed/ fruit weight resulting in a higher yield compared to the untreated control. Fageer and Ahmed (2008) studied the impact of infestation of the green pit scale insect on production costs and productivity. Their finding revealed that, the proportion of farmers who produce less than half a sack/ tree/ year (about 50 kg) is 68% of the total farmers in the study area due to the infestation by the green scale insect and weak palm care.

The date palm treated with the high doses of thiamethoxam and imidacloprid, started re-growing, which was a clear indication that the pests damage had stopped and the palms were no longer under stress. This phenomenon was independent of the pesticide formulation applied to the palm. The increase in number of the scale pest on control and treated trees was slower in the trees treated with the higher doses compared with the lower doses, and these agreed with results obtained by Kehat *et.al* (1967) which stated that the chemicals that injured the females of the green date palm pit scale insect had a prolonged effect in preventing the reappearance of the young nymphal instars and thus scale insect population were renewed only at a much slower pace. It was observed that the treated trees remained free from termites, white scale insects and ant's infestation

The efficiency of imidacloprid (0.35, 0.70 and 1.05 g a.i./plant) in controlling citrus leaf miner (*P. citrella*) infesting lemons was determined in a field experiment conducted in Argentina. Application of 0.35 g a.i. imidacloprid/plant controlled citrus leaf miner up to 100 days after planting (Salas and Goane, 2003).

The principal sucking pest is the date palm cicada (*Ommatissus binotatus libycus*). The plant is damaged not only by the insect's feeding activity but also by its honeydew, which is colonized by fungi, and traps dust, thus reducing the tree capacity for Photothynenthis. The yield was also reduced by varies species of scales and mealy bugs. Very good control can be achieved with soil application of imidacloprid or thiamethoxam (Al-Jboory *et.al*, 2001, Ahmed *et.al*, 2002, 2004 and 2007).

A comprehensive program by Plant Protection Directorate (PPD) has been conducted during (2008- 2016) in six locations Elgaba Scheme , El Golid area, Old Dongola ,Elburgaig scheme in Northern State , and in Abuhamad area in River Nile State and also in Khartoum State. the insecticides used and their quiantaty are presented in table (1)

1.2.Trunk injection method

Results indicated that, trunk injection was an effective and reliable method for controlling the green pit scale insect (Ahmed, 2004). The higher dose treatments; Actara (10g), Rinfidor (20ml), Comodor (20ml) and Confidor (20ml) were superior to the lower doses and the untreated control in number of total dead insects and percentage mortality ,even 12 weeks after application (the last count). Results of percentage mortality of adult females and immature stages showed the high efficacy of insecticides as reflected by the hundred percent mortality of adult female and immature stages during the second week after injection (Fig 5&6).

The same finding were obtained by Joseph *et.al* (2003 and 2007) when they test a trunk Micro-infussion of IMA-jet (imidacloprid) for control the Hemolock Woolly Adelgid (a tiny, piercing and sucking insect) that feed on Hemolock twings. Results indicated that, Adelgid mortality may occur with 14-28 days and continue for up to 2 years. Hemolocks respond to treatment with a resumption of growth. On the other hand, Smitly *et .al* (2006) success in controlling Emerald Ash Borer (*Agrilus planipennis* Fairmaire) infesting green Ash trees (*Fraxinus pennsylvanica* Marsh) with trunk injection using either imidacloprid (Mauget imidide and Arborjet IMA-jet), or orthen (Acecaps). Resluts indicated that the Arborjet trunk injection treatments with imidacloprid provided a high level of control (92-100%). Acecaps trunk injection containing acephate, gave 76% control.

Fernandes Cordova and Gallego (1997) found that oaks infested by oak scale insect were cured by injection with prepared capsules of 225ml Acephate or imidacloprid solution, these insecticides were effective in controlling the scale pest, moreover, they pointed out that grater than 79% control of oak scale was obtained when acephate and imidocloprid were injected at rates of 7.5g a.i. and 0.8ml per tree respectively. Mathen and Kurian (1977) pointed out that sevin at a concentration of 1% injected in coconut trunk cased 93% reduction in the infestation level of red palm weevil.

The distribution rates of the thiamethoxam as Actara 25 WG 24 h. post injection of 1 and 2 g a.i./palm (Al-Sammariae *et.al*,2006) showed that it distributed into the sap and it was detect in the injection side and also in the opposite side at different heights. The result indicated that thiamethoxam translocate rapidly into date palm trunk and reaches the leaves in a short time so it can be drown out of the findings that it can be employs as a fast chemical remedy against most palm insect pests.

As mentioned before, trunk injection requires the use of a systemic insecticide. It is a safe method which affects the pest only without any side effect on natural enemies. Thus the method causes little adverse effects on the environment, when wide spray application with contact insecticides using aircrafts and heavy machinery had been conducted in areas like El Golid the pest recovered within one year and spread from the target area to infest Elgaba scheme and Old Dongola (El Fahal *et.al*., 1993, Obied, 1997 and Ahmed, 2003). Furthermore, trunk injection protects the insecticide from adverse climatic factors. If we take into consideration that more than 60% of date palm trees in the Sudan are not irrigated, the use of trunk injection is very useful as an alternative solution to soil application method.

This method not only increases user's safety, it also allows the work to be carried out in an extremely economical manner. The dose used is decreased to less than 50% compared to soil application. On the other hand, a three- man team can do the work, one man boring the hole, the second inserting the tube into the holes and the third inject insecticides and close tubes. Date palms treated by trunk injection, continued to develop normally during the past four seasons. No phytotoxicity had been observed till now in the treated trees. No insecticide residues have been detected either in dates, soil or grasses.

Insecticides are applied through direct injection into the trunk of the date palm to control the red palm weevil (Oihabi, 2003). The influence of injector size, tree species, and season on uptake of injected solution, uptake volume varied among species and injector size, but it usually increased with time. Uptake volume usually decreased as injector diameter decreased. In nonresinous species, the 6 mm (0.24 in.) injector gave the best results, but the 4 mm (0.16 in.) and the 3 mm (0.12 in.) injectors also gave acceptable results. Rubidium content increased over time in sampled needles. One day after injection, Rb+ was recovered in all three sections, indicating a homogeneous distribution throughout the tree (Al-Jboory et.al 2001).

Results of residue analysis indicated that, no residues of both imidacloprid and thiamethoxam were detected in dates, soil and intercropped plants when treated with the high doses (Abbas et.a l2005 and Ahmed 2008and Sanhuri et al ,2017). It could be concluded that, chemical control is more rewarding compared to non control regarding productivity as well as net benefits

In addition to the direct benefits from using this package, farmers may increase their yield by cleaning and scheduled irrigation of their gardens (as the package recommended) and by saving irrigation water as a result of changing the traditional method of irrigation (flood method) to direct irrigation (using irrigation basins round the date palm trees) and then reduced the expected diseases which are active in humid conditions urce: PPD, Sudan 2015

2. Varietal susceptibility

The four Dates palm varieties under test showed different levels of infestation .Result in Fig.7 &8; indicate that Gondeilla variety was the most susceptible. The infestation of Barakawi and Jaw were moderate, and the least affected variety was Waddlagi. The results were constant over months and in the four locations.

The number of living insects (female and immature stages) was not affected by inter cropping or without inter cropping although the least susceptible variety Wadlagi showed less number of living insects in date palm farms with inter cropping (less than 100 scale inset per leaf) compared with without intercropping during the four months which recorded more than 300 scale per leaf .The results showed that the infestation is not affected with the direction or the position of the leaves on the tree, although the highest population of the scales were found on the lowest leaves and on the south west direction of the tree. Similar result was obtained by Ali (1989) in ElGolid area where, the varietal susceptibility studies showed that Moshrig and Gondeilla varieties were the most susceptible. Barakawi was moderately susceptible while Jaw and Tamoda were the least susceptible according to average number of scales per leaflet.

All the variety studies in the Sudan were conducted on white scale. Nasr (1982) reported that, in the Sudan, Medina and Wad-Katib varieties sustained the highest number of white scales per leaflet .The least susceptible variety was found to be Jaw. The susceptible varieties were those having broader leaflets. Osman (1992) reported that Moshrig and Gondiella varieties have comparatively less number of leaflets per frond, followed by barakawi and Jaw, while Tamouda possessed the highest number. Ali (1989) stated that the Gondiella and Moshrig have comparatively larger size of leaflets, followed by Jaw and Barakawi, while Tamouda possessed the smallest size of leaflets .Mohamed (1991) concluded that, the most susceptible varieties were those having comparatively less number of leaflets per frond and a larger size of leaflets. As indicated by Alhafidh *et al.* (1981) this character may influence the degree of light intensity and other micro-environmental factors.

The ongoing research (Ahmed, 2017) indicate that about six of introduced date palm cultivars including Sukkari, khilas, Barhi, Anbara, khadari and nabot sief were planted in the infested area (Algaba Scheme, since 2006), observed different tolerance to infestation, and the fruits of these varieties under test, reached maturity without any loss, this may focus the important of these varieties in any strategy for date palm plantation in infested areas in future.

3. Natural enemies' associated with the green pit scale insect in the Northern State, Sudan.

The results showed that there were two species of predatory Coccinellids associated with date palm green pit scale insect in the different locations. The first was *Cybocephalus dudichi* and the second was *Pharocymnus numidicus* (plate 5 d). They were known as predators of white scale insects but their abundance with this insect show that they are general predators. The monthly mean total number of the adults of the two species was recorded as shown in Fig. (9) and Fig. (10) for *Cybocephalus dudichi* and *Pharocymnus numidicus*, respectively. The higher numbers were observed in months when the green pit scale was active (Fig. 8). The number of these species was low in winter (November – December); it was affected by the low number of the green pit scale insect. Fig. (4) Shows that the number was not affected in date palms orchards with or without intercropping. Other species were recorded like *Chrysopa spp.* and a predatory mite. Some adult females were attacked by a parasite because exit holes were found in the covers of the scales. Also some adult females became black with a black liquid like polyhydrosis.

The survey revealed the existence of two species of predatory Coccinellids associated with date palm green pit scale insect in the different locations. The first was *Cybocephalus dudichi* and the second was *Pharocymnus numidicus*. They were known as predators of white scale insects (Schmutterer, 1969., Nasr, 1982 and Ali, 1989) but their abundance with this insect show that they are general predators. The monthly mean total number of the adults of the two species was recorded for the two species. The higher numbers were observed in months when the green pit scale was active. The number of these species was low in winter (November – December); it was affected by the low number of the green pit scale insect.

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Dafalla and Hassan (2010) in Dongola Research Station success to isolate an endoparasite. The survey was made from August 2008 to July 2009 in the Northern State and it covered the localities of Artigasha Island, Dongola, Old Dongla Elburgag, Elgaba, Elgolid, and Urbi. Date palm trees infested with the green pit scale insect were taken at random and marked with paint in each locality. The marked trees were monitored monthly for the pest and possible parasitism. Parasitoid exist hole in the scale (Fig1).

The survey revealed presence of parasitism in the green pit scale insect in all the surveyed areas. 1 to 16% parasitism was recorded. The overall mean parasitism was 9.2%.The parasitism existed through the year and was relatively high during January, February and March than in the other months. The parasitoid was identified as *Metaphycus* sp (Hymenoptera: Encyrtidae) and it is a primary endoparasitoid (Fig2). The identified parasitoid is a new record for Sudan and to their best knowledge not reported before in association with the green pit scale insect worldwide.

The *Metaphycus* spp. are among the most important natural enemies of many scale insects, commercially produced and frequently used in augmentative releases to control several species of scale insects (Flint *et al* 2010, and Schweizer *et a*l 2002) Conservation of this newly detected parasitoid in the date palm habitat in the Northern State is important. Further detailed studies on the parasitoid are necessary to initiate biological control of the green scale insect on date palm in the Sudan.

None of the natural enemies associated with the scale *Asterolecanium phoenicis* Rao, was reported before 1967, even in catalogues of parasites and predators (Thompson, 1950; Thompson and Simmonds, 1964). Kehat (1968) reported that females and nymphs of the pit scale, *Asterolecanium phoenicis* Rao. were highly resistant to attack by the Coccinellid predator, *Pharoscvmnus numidicus* Pic. due to their hard scale coverings.. Yinon (1969) reported that the Asterolecaniids, the soft scales, the mealy bugs in addition to the armored scales, constitute the host range of the Coccinelled predator, *Chilocorus bipustulatus* L. The intensity of predation on the *Asterolecanium phoenicis* Rao. was only one-third the intensity of predation on the armored scale insects.

In Sudan, Ali (1989) reported that, no specific natural enemies were observed associated with this pest. However ,some females were observed attacted by an endoparasite which failed to emerge from the scale but the ongoing research in Dongola Research Station success to isolate this parasite and there were attempts to idindificate and studing the feeding capacity .An unidentified mite was found associated with both the green date scale and the white palm scale .The catalogues of Thompson (1950) and Thompson and Simmonds (1964) reported parasites and predators on other species of the genus *Asterolecanium* with varying degrees of parasitism and predation in some biological control attempts that were carried out. The majority of the natural enemies reported were considered inefficient and some were reported to have reduced value.

In general, scales are often well controlled by natural enemies, especially when predator and parasite activities are not disrupted by ants or applications of broad-spectrum insecticides such as Carbaryl, Malathion, or pyrethroids applied to control other pests. (Carvalho *et al.*, 2006)

Depending on the extent to which biological control has been disrupted, it may take several months of conservation efforts before scale populations are reduced by biological control. If current levels of scales are intolerable, it is recommended to use a short residual low toxicity compound such as oil or insecticide to reduce scale populations while conserving natural enemies which is the strategy recommended by Dongola Research Station.

CONCLUSION

1. Date palms are of major socioeconomic and social importance in the Northern State,
2. Date palm is still grown in Sudan by congenital methods without attention to irrigation, fertilization, or other cultivation practices.
3. Date palm production and plantations considerably deteriorated in the last years as a result of biotic and a biotic stresses, among which the green pit scale is the most important.
4. Soil application and trunk injection of imidacloprid (Confidor 200SL, Rinfidor20% SL, Comodor 20%, Zathron 20%SLand Sinfidor 20%SL) and thiamethoxam (Actara 25WG) were Highly effective in controlling the green pit scale insect.
5. They proved to be very effective as a protective measure against new infestation.
6. The two methods of application do not require any expensive machinery or labour for application. They can be safely applied.
7. Trunk injection truly effective and reliable method for controlling the green pit scale insect, with minimal environmental impact.
8. The two methods of application are highly economical and safe for the user and appear to be safe for the beneficial insects.
9. Date palms treated with different insecticides using the two methods, developed normally during four seasons. No phytotoxicity has been noticed in the treated trees.
10. The tested insecticides checked termites and many other pests, but did not affect
11. Cultivation of Wadlagi variety is recommended in the infested areas.
12. Further studies are needed to study the susceptibility of introduced soft and semi soft, date palm varieties to green scale infestation.

13. The current surveys indicated that there is sign of natural enemies (predators and parasites) of this pest.
14. The effort of research in Dongola Research Station (ARC) success to isolate an endoparasite identified as *Metaphycus sp.*
15. Further studies are needed to confirm the efficacy and mass rearing of the natural enemies.

REFERENCES

Abbas, Ihsan. A., EL Habieb, Rawda Y. and Gaafer A. EL Zorgani (2005). Residues of imidacloprid compounds (Confidor 200 SL, Rinfidor 200SL and Comodor 200 SL) in Date palms *The Proceeding of the 72nd Meeting of the Pests and Diseases Committee June, 2005, Agricultural Research Corporation, Wad Medani Sudan.*

Ahmed, M. A. (2001). Susceptibility of some date palm varieties to green pit scale insect infestation, ARC, Palm Research Program Annual Report 2000/2001.

Ahmed M.A. (2004): The efficacy of Confidor 200 SL against the green date palm pit scale insect (Asterolecanium Phoenicis Rao.) (Homoptera: Asterolecaniidae). The proceeding of the Second National Pest Management Conference in the Sudan, 6- 9 Dec. 2004. Faculty of Agricultural Sciences University of Gazira, Sudan.

Ahmed, M.A (2005). Recommendation of four systemic insecticides for control of the green date palm pit scale insect (Asterolecanium Phoenicis Rao.) (Homoptera: Asterolecaniidae), using two method of application. Proceedings of 67th Meeting of the Pests and Diseases Committee, ARC Conference Hall, Wad Medani, Sudan, June 2002.

Ahmed, M.A (2008)). Efficacy four systemic insecticides for control of the green date palm pit scale insect (Asterolecanium Phoenicis Rao.)(Homoptera: Asterolecaniidae), using two method of application.. P.h.D. Thesis, Faculty of Agriculture, Universityof Khartoum, Sudan.

Ahmed, M.A; Oman A.M. and Makawi H.M. (2002). Recommendation of Confidor 200 SL (imidacloprid) for control of the green date palm pit scale insect (Asterolecanium Phoenicis Rao.) (Homoptera: Asterolecaniidae). Proceedings of 67th Meeting of the Pests and Diseases Committee, ARTC Conference Hall, Wad Medani, Sudan, June 2002.

Ahmed, M.A; S.Gubara,S. Sanhuri K.gebrel and I Siddig (2013). The efficacy of two systemic insecticides against the green pit scale insect (Palmapsis phoenicis Bodenheimer (Green)) (Homoptera: Asterolecaniidae) infesting date palm in Northern Sudan Proceedings of 72nd Meeting of the Pests and Diseases Committee, ARTC Conference Hall, Wad Medani, Sudan, December, 2013.

Alawi, H.A. (1993) Analytical Method and Results "Date samples" Faculty of Science, U. Of Jordan, Amman-Jordan.

Ali, A.A. (1989). Studies on Astrolecanium phoenicis (Rao). A date palm scale insect in El Golid area. MSc. Thesis Faculty of Agric. U.of K.

Ali, A.A. and Tibin, A.B (1992). Chemical control trials against termites and green pit scale insect in Dongola Province (Pest and Disease Committee Annual Meeting Sep.1992) ARC HQ, WadMedani, Gezira Sudan. J. 4 (2): 247 – 266.

Ali, G. A.; Karbil, A.K. and M. Ibn Aouf (1998). Survey on Production, Handilling, Storage and Marketing of Dates in the Northern State, Sudan.In Proceedings of the First Conference on Date palms (8-10 March, 1998). Al- Ain Hilton Hotel, Al-Ain, U.A.E.

Baballa, A., M (2002).The characterization of dry Al-Jboory, I.J.: A.I. Al-Sammariae; J.F. Whaib and W. A. Ahmed (2001). Evaluation of Thiamethoxam in a different Application Techniques to Control Dubas (Ommatissus lybicus (Bergevin). Arab Journal of Plant Protection 19 (2): 107-112.date palm in the River Nile State.Msc. Thesis, Unversity of Wadi Elneel, Sudan.

Carvalho, G. A., Moura, A. P., Bueno, V. H. P (2006). Side effects of pesticideson Trichogramma pretiosum (Hymenoptera: Trichogrammatidae) Bulletin OILB/SROP, Vol. 29, No. 4, pp. 355-359.

Daffalla E. Yousof and Ahmed H. Mohamed (2010).Metaphycus sp (Hymenoptera: Encyrtidae), a parasitoid of the green pit scale insect on date palm in the Northern State, Sudan: A new record. The 82nd Meeting of the Pests and Diseases committee. ARC, Wad Madni, June 2010.

El Fahal, O. A. (1993). Life System Analysis of Paralatoria blanchardii Targ. In the Northern State of Sudan. P.h.D. Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

El Hassan, E., M. (2006). Date Palm Diseases in Merawi and Debba Areas, in The Northern State, Sudan .Crop protection Research Center Annual Report, 2006/2007 Agricultural Research Corporation (ARC) Sudan

Ezz, I, A (1973). Asterolecanium phoenicis (Homoptera-Coccoidea), a date palm pest recorded for the first time in Egypt. Agricultural Research Review, Vol. 51, No. 1, pp. 47.

Ezz, A.I.; Abu El Ezz, A. (1961). Asterolecanium phoenices Rao. Infesting date palm in Saudi Arabia. Bull. Soc. Ent. Egypt XLV. 45:407.

FAO. (2002): Date Palm Cultivation .Edited and compiled by Abdelouahab Zaid and. Arias - Jimenez, E.J Date Palm Research and Developmen Programme . United Arab Emirates. FAO Plant Production and Protection Paper, No. 156 - 285 PP.

FAO. (2010): Agro-Statistics Database (http://faostat.fao.org/site/340/default.aspx).

Flint, M. Lowise, Steve H. Dreistadt, and Jack K. Clark. (2010).Parasitesof Arthropods In: Natural Enemies Handbook: The illustrated guide to biological control.p.64. http// books. Google .com. pp. 55-66.

Harten A.V. and AbdelarhmanA.A. (1996). Biological control of scale insect on date palm in Northern Sudan. A report on behalf of GTZ project, Sudanese German Services for vegetable and fruit farmers (SVFF).

J. R. Faleiro (2017). Report of Red Palm Weevil Mission to Sudan. On Behalf of'Khalifa International Award for Date Palm and Agricultural Innovation' The first International Sudanese Date palm Conference December, 2017 Khartoum, Sudan.

Felippe, M. R., Garbim, L. F., Coelho, J. H. C., Ximenes, N. L., Sanchez, A. L. , Yamamoto, P. T. (2005). Chemical control of orthezia in citrus. Laranja, Vol. 26, No. 2, pp. 251-264.

Felippe, M. R., Garbim, L. F., Coelho, J. H. C., Ximenes, N. L., Sanchez, A. L. , Yamamoto, P. T. (2005). Chemical control of orthezia in citrus. Laranja, Vol. 26, No. 2, pp. 251-264.

Kehat, M. (1967). Survey and distribution of ladybeetles (Coleoptera: Coccinellidae) on date palm trees in occupied Palestine. Entomophaga, 12(2): 119 – 125.

Joseph, J.D.; P., M.Wild; L.Ramasamy; P. casstillo and Cherstine, T. (2003). Efficacyof Arborjet Viper Micro-injections in the management of Hemolock Wolly. Journal of Arboculture .29(1)327-330.

Joseph,J.D.;E.J.,BRISTOL;S.D.Sifleet;Joseph L. and P.,M.Wild(2007).Efficacy and Duration of Trunk –injected imidacloprid in the Management of Hemolock.Aborculture and Urban Forestory .33(1)12-21.

Kehat, M. (1968). The feeding behaviour of Pharoscymnus numidicus (Coccinellidae), predator of the date palm scale Paralatoria blancharidiiTarg.Ent.Exp.App., 11:30– 42.

Mathen, K. and Kurian, C.(1977). Sevin controls red palm weevil at low cost. Coconut Bull., vol. 1 (5): 7 – 8

M. M. A. Khairi (2015). Date palm Status and Prospective in Sudan. Chapter 5 in Date Palm Genetic Resources and Utilization, J.M.Al- khayri (eds).Volume 1:Africa and the Americas . D01 101007/978-91-017-9694-1-5.Springer Science + Business. Media Dordrecht, 2015.

Mohamed, S, G (1991).Studies on the date palm scale isect Palatoria blanchardi Targ. (Homoptera:Diaspididae) Msc thesis Faculty of Agriculture U.of K

Nasr, O.E. (1982). Studies on some natural enemies of Diaspin scale with special reference to Parlatoria blancharidii Targ. MSc. Thesis .faculty of Agric. University of Khartoum.

Osman, A. M. A. (1992). Date Palm Production in Sudan. Report to National Research Council, Khartoum, Sudan.

Osman, A. M. A. (2001). Development of date culture in Republic of Sudan. Paper presented at a workshop on date palm culture and date production in Republic of Sudan. Date Palm Research and Development Net work, Khartoum, Sudan22-17 Augest2001.

Oihabi ,A. (2003). Major pests of the date palm in The Date palm from traditional Resource to green wealth, The Emirates Center for Strategic Studies and Research ECSSRE

Salas, H. , Goane, L.(2003) .Evaluation of different doses of systemic pesticides applied before definitive planting to control the citrus leaf miner Phyllocnistis citrella Stainton. Revista Industrial y Agrícola de Tucumán, 2003, Vol. 80, No. 1/2, pp. 33-36, (English abstract).

S. sunhori , Mahdi A. A., S. Gobara I., A.M. Osman and I. A. Siddig (2017). The efficacy of Sicofidore 30.5% against the green pit scale insect (Palmapsis phoenicis Bodenheimer (Green)) (Homoptera: Asterolecaniidae) infesting date palm in Sudan .The 96th Meeting of the National Pests & Diseases Committee ,June, 2017) ARC HQ, WadMedani, Gezira Sudan

Schmutterer, H. (1969). Pests of crops in Northeast and Central Africa. Gusty Fisher Verlav Stuttgard.

Sherif M.A. (1994). Results of Confidor trials for the control of red date palm weevil Rhynchophorus ferruginous (Oliv.) in Dammam area of Saudi Arabia. Horticulture section and plant protection expert. Al Emar Group Est. Riyadh.

Schweizer, H.,Joseph G.Morse, Robert F. Luck and Lisa D.Forster (2002). Augmentative releases of a parasitoid (Metaphycus sp. nr. flavus) against citricola scale (Coccus pseudomagnoliarum) on oranges in the San Joaquin Valley of California. Biological Control.Vol. 24. Issue 2, 2.pp 153-166.

Shinwari, M.A.(1993). Date palm.In Encyclopedia of Food Science, Food Technology and Nutrition, Macrae, R.; Robinson, R .k. and Salder, M. (eds.) .Academic Press Ltd, London, UK, pp.1300-1305.

Smitley,D. R.;T. W. Davis andK.F. Newhouse (2006).Emeraled Ash borer efficacy test in South Troy. Aborjet Research Reports.Deparment of Entomology, Michigan State University.

Thompson, W.R. (1950). A catalogue of the parasites and predators of insect pests.Sec.1: Parasite Host catalogue Part 3; Parasites of Homoptera (2nd edit.) The commonwealth Bureau of biological control, Canada.

Thompson, W.R. and Simmonds, F.J (1964). A catalogue of the parasites and predators of insect pests.Sec.3: Predator Host catalogue .The commonwealth Bureau of biological control, Canada.

Yinon, A. (1969). Food consumption of the armoured scale lady beatle Chilocorus bipustulatus L Ent.Exp.App.12:139-146.

Zaid, A and Arias - Jimenez, E.J. (1999): Date Palm cultivation. FAO Plant Production and Protection Paper No. 156 - 285 PP.

Tables

Table (1). insecticides used and their quiantaty used by Plant Protection Directorate (PPD)

Year	Treated trees	Insecticide	Quantity /liter
2008	578068	Confidor + Actara	10035.9
2009	587517	Confidor	11670.9
2010	459136	Confidor	8073.75
2011	803578	Confidor	17584.5
2012	468754	Confidor+ Midas	8782.72
2013	459136	Confidor+ Midas	250.195
2014	408376	Confidor+Akropid+ Midas	8263
Total	376465		64660.995

Source PPD (2015)

Figures

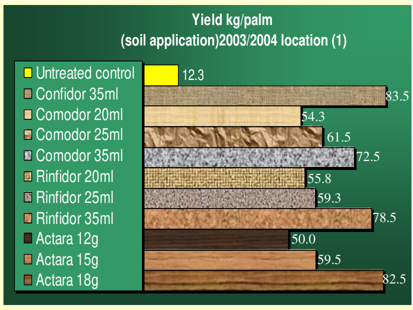
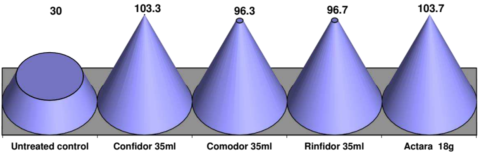


Fig. 1. Ahmed et al (2005)

Fig. 2.Ahmed et al (2005).

Yield kg/palm (soil application) 2003/2004 location (2)



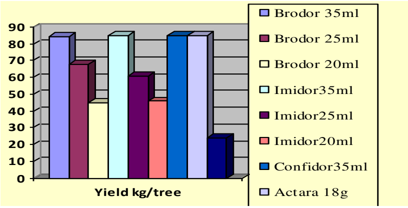


Fig .3. Ahmed et al (2013).

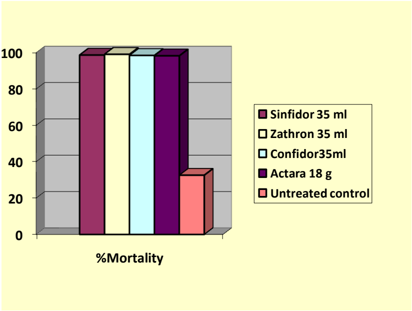
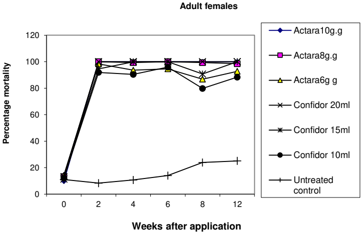


Fig. 4. Sanhuri *et al* (2017)



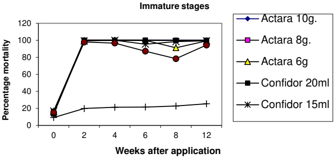
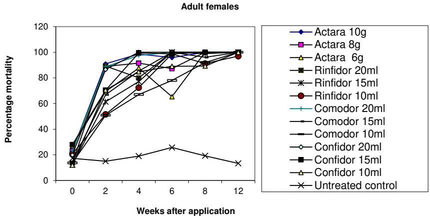


Fig 5: Mean biweekly percentage mortality of adult females and Immature stages of green pit scale insect from trees treated with trunk injected insecticides in Elgaba scheme season, 2003/2004.



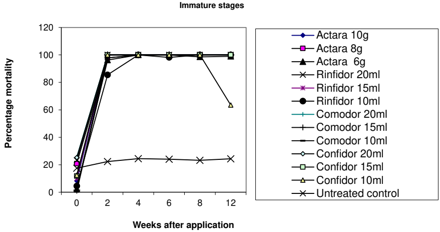
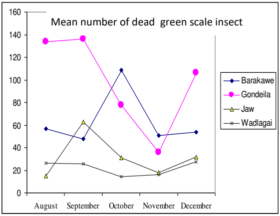
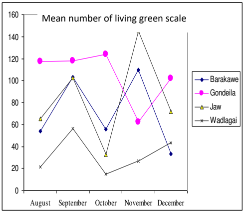


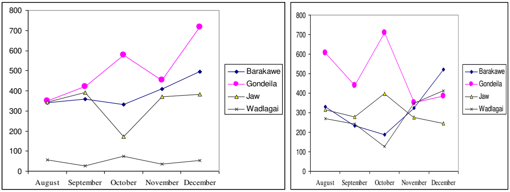
Fig. 6: Mean weekly percentage of adult females and immature tages of green pit scale from trees insect treated with trunk injected insecticides, El Golid, season 2004/2005.





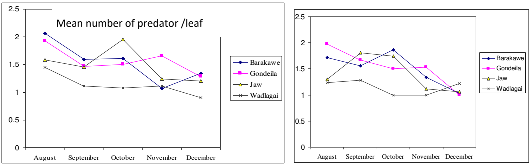
With intercropping　　　　Without intercropping

Fig.7. Mean number of the dead adults of the green pit scale insect on four date palm varieties (with and without intercropping) at the study (Ahmed 2008)



With intercropping　　　　　　　　Without intercropping

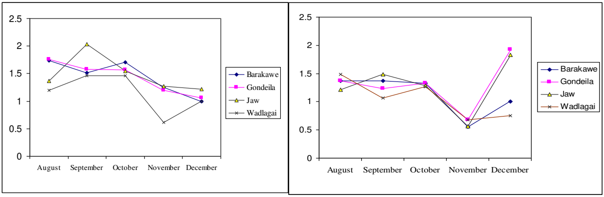
Fig.8: Mean number of the living adults of the green pit scale insect on four date palm varieties (with and without intercropping) at the study sites.



With inter cropping　　　　　　　　Without intercropping

Fig.9: Mean number of *Cybocephalus dudichi* on four date palm varieties (with and without intercropping) at the study sites (Ahmed 2008).

Mean number of predator /leaf



With inter cropping

Without intercropping

Fig.10: Mean number of *Pharocymnus numidicus* on four date palm varieties (with and without intercropping) at the study sites.(Ahmed 2008).

Metaphycus sp. a parasitoid of green pit scale insect on date palm, Daffalla and Hassan (2010)

White scale, *Paralatoria* parasitoid

Plate 3 Natural enemies (Daffalla &Hassan 2010,Ahmed 2008)

Source: Chilocorus bipustulatus L

Pharoscymnus numidicus
a predator of scale insects on date palm

Source: (Daffalla and Hassan 2010)

Source: (Ahmed 2008)

Use of 5-hydroxypipecolic acid as authenticity and biomarker for date palm fruit-based foodstuffs

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Abstract

In previous work [1] we had shown that edible date fruits contain large quantities of the non-proteinogenic amino acid 5-hydroxypipecolic acid (2*S*,5*R*-5-hydroxypipecolic acid or *trans*-5-hydroxypipecolic acid), abbreviated Pip(OH) in the following. Pip(OH) had been detected in in twelve named cultivars of date fruits of different origin. Results: We hypothesized that Pip(OH) might serve as reliable and specific marker for date fruits and, consequently, date fruit-based foodstuffs. In order to prove this hypothesis, we analyzed total hydrolysates of various date fruit cultivars of different maturation stages (*Khalal, Rutab, Tamar*), preparations of various date pastes and date syrups (*Dibis*), and commercially available date vinegars. Automated amino acid analysis based on ion-exchange chromatography was used as well as gas chromatography - high resolution mass spectrometry (GC-HRMS) of *N*(*O*)-pentafluorobutyryl-pipecolic acid-*O*-pentafluorobutyl esters. Complimentary, underivatized Pip(OH) was determined using hydrophilic interaction (HILIC) liquid chromatography (LC) combined with high resolution mass spectrometry (HRMS) performed in the electrospray-ionization mode. Conclusions: Presence of Pip(OH) was detected in all aforementioned foodstuffs, surpassing or approaching the most abundant protein amino acids in date fruits *i.e.* aspartic and glutamic acid, alanine, proline and leucine. Since Pip(OH) is rare in edible fruits this non-proteinogenic amino acid is proposed as novel authenticity or dietary biomarker for date palm fruit-based foodstuffs in general. **Acknowledgement: Research supported by National Plan for Science, Technology and Innovations (NPST) at King Saud University (project no. 11-AGR1600-02).**

Long-term assessment of the impact of salinity on fruit yield and quality in eighteen date palm varieties from the Arabian Peninsula

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Abstract

Date palm (*Phoenix dactylifera* L.) growth and production in the arid regions is adversely affected by increasing soil and water salinities. Although many aspects of date palm nutritional values, physiology, genetics and many more are extensively researched, the evaluation of salinity impact on date palm growth and productivity are limited to short duration studies and focused on seedling stage mostly. A large gap in understanding salinity impact on date palm is therefore very evident. To address the complex issue of the impact of salinity on date palm trees, the International Center for Biosaline Agriculture (ICBA) located in the UAE, established a long-term experiment using eighteen local and regional date palm varieties from the Arabian Peninsula on nearly 2.5 ha in 2001 and 2002 on its experiment station to evaluate the impact of three levels of salinity (5, 10 and 15 dS/m ECW) on date palm productivity. Long-term results showed that fruit yield at Rutab and maturity stages were severely reduced at high salinity based on 8 years of yield data. Rutab fruit yield ranged from 67-146 kg/tree at the low salinity, while at the high salinity (15 dS/m) ranged from 21-98 kg/tree. At maturity, fruit yield reduction at the high salinity (15 dS/m) relative to the low salinity reached 67%. The 18 date palm varieties were widely different in the impact of salinity and yield stability. Regression fitting and Principal Component Analysis led to the identification of four different groups of date palm varieties with relation to salinity tolerance and thresholds. Group 1, including varieties Lulu and Barhi, are high yielding varieties with high salinity tolerance. Average salinity level at 50% yield reduction is 12 dS/m. The second group includes varieties: Khisab, Sukkari, Jabri, Shahlah, characterized by high yielding varieties with sensitivity to increase in salinity, yield decline highly at high salinity. Average salinity level at 50% yield reduction is 10 dS/m. The third group includes varieties: Farad, Um Al-Hamam, Naghal, Abu-Maan, Rhothan, and characterized by Medium to high yield potential under low salinity with moderate salinity tolerance. Average salinity level at 50% yield reduction is 9 dS/m. The fourth group includes varieties: Shagri, Khnizi, Nabtat Saif, Ajwat Al-Madinah, Khalas, Maktoumi, they have low yield potential with low salinity tolerance. Average salinity level at 50% yield reduction is 8 dS/m. The previously reported limits were shown to be higher than the findings of this work. Initial results showed that under irrigation with high salinity water, the actual water use by the plants declined by 40-50%. Such findings have great implications for water management under saline conditions. The results and findings will help better understand the impact of salinity on date palm and in guiding further work on genetic enhancement and the development of integrated management approaches for date palm under marginal conditions.

Use of high hydrostatic pressure as an alternative preservation method for fresh dates

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Abstract

The effects of high hydrostatic pressure (HHP) treatments on microbial contamination, chemical and physical properties of fresh dates (mature full colored Bisr) was studied. Khalas, Barhi and Hilali cultivars were treated at 200, 250, 300 and 350 MPa using HHP research apparatus. The objective of such treatments was to preserve fresh dates without adversely affecting its properties. Treating fresh dates at 300 MPa for 5 minutes at 40°C reduced microbial contamination in about 2.5 log cycles. Applying 250 MPa was enough to control fresh dates contamination with molds, yeasts and coliforms. Both treatments were enough to reduce fresh dates microbial contamination to acceptable levels. HHP caused no significant effect on fresh dates chemical properties (moisture, sugars, protein, pectin and acidity). However, a slight decrease in moisture contents due to HHP was observed. Fresh dates lightness (L*) significantly decreased due to application of HHP. Only fresh dates treated at 300 MPs gave lower redness (a*) values compared with untreated sample. The effect of 300 MPa on increasing yellowness (b*) was observed for Barhi and Hilali but decreasing for Khalas. Hardness of all fresh dates cultivars significantly decreased as a result of HHP application. In fact, pressure applied at 300 MPa had adverse effect on texture, which may limit its suitability for use in fresh dates preservation.

Keywords: high hydrostatic pressure, fresh dates (Bisr), microbial contamination, color, texture.

Effect of Using Nano -Boron Versus Normal –Boron on Fruiting of Barhy Date Palms

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Abstract

This study was conducted during 2016&2017 seasons to examine the effect of using nano- boron at 0. 0125 to 0.1 % versus normal- boron in the form of boric acid at 0.025 to 0.2 % on fruiting of Barhy date palms grown under Minia region conditions, Egypt. Treating the palms with both nano-boron or normal-boron at the previous concentrations had an announced promotion on growth, palm nutritional status, yield and fruit quality compared to the control treatment.

Using nano –boron at the lower concentrations namely 0.0125 and 0.025 % considerably was Superior than using the higher concentration of normal-boron namely 0.1 and 0.2 % on promoting all parameters. Using nano –boron at concentration higher than 0.0125 % failed to show measurable effects.

For promoting yield and fruit quality of Barhy date palms grown under Minia region conditions, Egypt, it is recommended to spray the palms three times with boron via nano system at 0.0125 %.

Keywords: normal-boron , nano-boron ,Barhy date palms growth, yield, fruit quality.

INTRODUCTION

Nano technology system is a new alternative method versus traditional method for application of nutrients.

It has numerous advantages for facilitating the uptake of nutrients by plants (Al – Amin – Sadek and Javasuriya , 2007)

Msing boron via normal (Abdalla , 2008 , Mahoud , 2016 , Risk , 2017 Hassan , 2018) and via Nano (Refeai , 2014 , Roshdy and Refaau , 2016 , Wlassal et al., 2017 , Abdalla , 2018 and Mohamed , 2018) was very effective in improving growth yield and fruit venality in different fruit crops .

The target of this study was examining the effect using boron via Nano technology versus traditional method on growth and fruiting of Barhy date palms.

MATERIALS AND METHODS

This study was carried out during 2016 and 2017 Seasons in a private date Palm orchard situated at went Samalout , Samalout district , Minia Governorate on twenty – seven 12 years old Barhy date Palms the uniform in vigour date palms are planted at 10 x 10 meters apart . Surface irrigation system was followed the texture of the soil is clay Hand pollination was a achieved (table 1) inserting eight fresh male strands into the center of female spathe. Number of bunches per palms was adjusted to tem bunches and leaf bunch ratio was maintained at 8: 1 The select palms received the normal and common horticultural practices that are already applied in the orchard .

This experiment included the following nine treatments :

- Control .
- Spraying normal – boron at 0.025 % (0.25 g/L) .
- Spraying normal – boron at 0.05 % (0. 5 g/L) .
- Spraying normal – boron at 0.01 % (0.1 g/L) .
- Spraying normal – boron at 0.2 % (0.2 g/L) .
- Spraying normal – Nano – boron at 0.0125 % (0.125 g/L) .
- Spraying normal – Nano – boron at 0.025 % (0. 25 g/L) .
- Spraying normal – Nano – boron at 0.05 % (0. 5 g/L) .
- Spraying normal – Nano – boron at 0.1 % (1.0 g/L) .

Each treatment was replicated three times, one palm per each .

Normal boron was used in the form of bark acid (17 % B). Nano and normal boron were suayed three times before hand pollination gust after fruit setting (Last week "m/cd Feb." of Apr.) and at two months later (last week of Jane) triton B as a wetting a fruit was added to, all solutions of boron at 0.1 %. Spraying was alone till run off Randomized complete block design was followed.

During both sear our, the following measurements were recorded.

1- Leaf area (Ahmed and Morsy, 1999).
2- Percentages of N, P. and K in the pinnae (Wilde et al., 1985).
3- Tiled / palm (kg.) and bunch weight (kg).
4- Fruit quality parameters namely weight (9) height and diameter (cm) of fruit , percentages of seeds and pulp (T.S.S %) total , reducing and non – reducing , sugars % (Lane and Eynon , 1965 and A. O.A.C. , 2000) , total aridity % (as , maleic) acid (100 pulp) , total made fiber and total soluble tannius % (A.O.A.C, 2000) .

Statistical analysis was done (Mead et al. 1993). Treatment means were compared using new L.S.D parameter at S% .

RESULTS & DISCUSSION

1. Leaf area and it content of N , P. and K .

Date in Table (2) clearly show that treating Barhy date Palms three times with boron via normal at 0.025 to 0.2 % or via Nano at 0.0125 to 0.1 % significantly was followed by enhancing the leaf area and percentages of N, P and K , in the leaves relative to the control . Missing boron via Nano method was significantly superior than using at via normal source in stimulating the leaf area as well as N, P. and K, in the leaves, using normal boron at concentrations above 0.1 %. And Nano- boron above 0.025 % had meaning less promotion. Three result were true during both seasons.

2- Yield / palm and bunch weight Date in table (2) obviously reveal that supplying the palms with boron via bath methods (normal or Nano forms) had significant promotion on the yield / palm and bunch weight relative to the control. The promotion was corrected with increasing concentrations of boron applied via normal on via Nano system. Using boron via Nano system was significantly preferable than using it through conventional method in improving yield / palm and bunch weight. Increasing concentrations of boron applied via normal from 0.1 to 0.2 % as well as boron applied via Nano from 0.025 to 0.1 % failed to show significantly promotion on the yield and bunch weight from economical point of view , it is recommended to spray boron , in Nano form at 0.025 % to obtain the highest yield and bunch weight under such promised treatment yield / palm reached 166.0 & / 167.0 kg during both season , respectively . The untreated palms produced 100 & 99.0 kg during 2016 & 2017 season, respectively. The percentage of increment on the yield due to using the previous promised treatment over the control reached 66.0 and 68.7 % during both seasons , respectively , There result were true during both seasons .

3- Quality if the fruits :

Data in tables (3 & 4) obviously reveal that treating Barhy date palms with boron via normal method at 0.025 to 0.2 % as well as via Nano form at 0.0125 to 0.1 % was significantly very effective in improving quality of the fruits in terms of increasing fruit weight and dimensions , palp % , T.S.S % , total and reducing sugars % and decreasing seeds % , total acidity % , total fiber % and total soluble tannins % relative to the control . The promotion was associated with increasing concentrations of boron applied via both forms .Using boron above 0.1 % in normal form and above 0.025 % in Nano source had negligible promotion on fruit quality using boron via Nano-system was significantly favorable than using it via normal method in enhancing quality of the fruits.

The best results were obtained due to using boron via Nano system at 0.025 %. These results were true during both seasons.

DISCUSSION

The beneficial effect of boron on the biosynthesis and translocations of sugars natural hormones uptake of nutrients and water could explain the present results.

These results regarding the promoting effect of Nano-boron on growth yield and fruit quality of Barhy date palms are in harmony with those obtained by Refaai (2014), Roshdy and Refaai (2016), Wassel et al., (2017) and Abdalla (2018) the result Mohamed (2016), Risk (2017) and Hassan (2018) confirmed the beneficial effects of using boron via normal method on grow with and fruiting of Barhy date palms.

CONCLUSION

Carrying jut three symays of Nano boron at 0.025 % gave the best result with regard to yield and fruit quality of Barhy date palms growth under Minia region conditions , Egypt.

REFERENCES

Abdalla, A.A. (2008): Behavior of zaghloul date palms to some pollen carriers and boron. M.Sc. Thesis Fac. Of Agric Minia Univ. Egypt.

Abdalla, O.G. (2018): response zaghloul date palms to the use of some microelements through nanotechnology M.Sc. Thesis Fac. Of Agaric Minia Univ. Egypt.

Ahmed , F.F. and Morsy , M.H. (1999) . A new method for measuring leaf area in different fruit species Minia J. of Agric. Res. & develop Vol. (19) Pp. 97- 105 .

Al-Amin Sadek, M.D and Jayasuriya, H.P. (2007) : Nanotechnology prospects in agricultural context . Proc. Of Inter. Agric Eng. Conf. 3.6 Dec. Banghok Ps 48.

A.O.A.C. (2000) : Official methods of Analysis 1sth Ed.

A.O.A.C. Washing ton , D.C. USA , PP. 490- 510

Hassan, H.S.T. (2018): Effect of spraying calcium, boric acid and silicon on growth yield and fruit quality of Balady mandarin trees Ph.D. thesis fec. Of Agric. Minia a Univ. Egypt.

Lane, H. and Enyon L., (1965) : determination of reducing sugars by means of Fehling's solution with methylene blue as indicator A.O.AC Washington D.C.U.S.A pp. 490 – 510 .

Mahmoud, Kh, M.H. (2016) : Response of Balady mandarin trees to foliar application of boron and silicon .
Ph.D. Thesis Fac. Of Agric. Minia Univ. Egypt.
Mead, R., Curnow, R.N and Hearted, A.M. (1993): Statistical Methods in Agricultural and Experimental Biology 2nd Ed. Chapman & Hall London, pp. 10- 44.
Mohamed, M.M.A. (2018): Physiological studies on Fertilization of Flame sunless grapevines by Nano technology system. M.Sc. Thesis Fac. Of Agric. Minia Univ. Egypt.
Refaai , M.M. (2014) : Response of zaghloul date palms grown under Minia region conditions to spraying wheat seed spurt extract and Nano boron stem cell s (4) : 22 – 28 .
Rizk, M.N.S. (2017): The beneficial effects of using silicon with some nutrients on fruiting of Zaghloul date palm, Ph. D. Thesis Fac. Of Agric Minia Univ. Egypt.
Roshdy, Kh., A. and Refaai , M. M. (2016) : Effect of nanotechnology fertilization on growth and fruiting of zaghloul date palms J. Plank production Mansoura Univ. Vol. 7 (1) : 93 – 98 .
Wassel A.M.M. , El- Wasfy , M.M.M. and Mohamed , M.M.A. (2017) : response of flame seed lens grapevines to foliar application of Nano fertilizers . J. product. & Dev. 22(3): 469 – 485.
Wilede, S.A., Corey, R.B., lyre , J.G. and Voigt GG.K. (1988) : Soil and plant analysis for tree culture 3rd Ed. Oxford and IBH publishing co. New Delhi India pp. 529 – 546.

Tables

Table (1): Analysis of the tested soil:

Parameter	**Values**
Sand %	8.5
Sielt %	10.0
Clay %	8.5
Texture	Clay
PH (1 : 2.5 extract)	7.8
Ec (1 : 2.5 extract) mmhos / cm	0.96
Total CaCo %	1.95
Total N %	0.09
Available P(PPM)	4.1
Available K (PPM)	409.3

Table (2) : Effect of using nano- boron versus normal – boron on the leaf , percentages of N. P and K, yield / Palm and bunch weight of Barhy of ate palms during 2016 & 2017 season .

Treatment	**Leaf area 2 (m)**		**Leaf N%**		**Leaf P%**		**Leaf K%**		**Tield / palm (Kg.)**		**Bunch weight (Kg.)**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Control	0.67	0.64	1.67	1.66	0.20	0.19	1.19	1.20	100.0	99.0	10.0	9.9
Normal-B at 0.025 %	0.73	0.69	1.74	1.73	0.26	0.26	1.24	1.25	120.0	121.0	12.0	12.1
Normal-B at 0.05 %	0.79	0.75	1.81	1.80	0.32	0.29	1.29	1.30	130.0	132.0	13.0	13.2
Normal-B at 0.1 %	0.86	0.82	1.88	1.86	0.36	0.33	1.34	1.36	170.0	143.0	14.0	14.3
Normal-B at 0.2 %	0.87	0.83	1.89	1.87	0.37	0.34	1.35	1.37	143.0	144.0	14.3	14.4
Nano – Bat 0.0125 %	0.93	0.90	1.95	1.93	0.43	0.39	1.41	1.42	155.0	156.0	15.5	15.6
Nano – Bat 0.025 %	1.00	0.46	2.00	2.00	0.47	0.44	1.46	1.47	166.0	167.0	16.6	16.7
Nano – Bat 0.05 %	1.01	0.96	2.01	2.01	0.48	0.45	1.47	1.48	169.0	169.0	16.9	16.9
Nano – Bat 0.1 %	1.02	0.47	2.02	2.02	0.49	0.46	1.48	1.49	171.0	170.0	17.1	17.0
New 1- S. Dat 5 %	0.06	0.05	0.05	0.06	0.04	0.03	0.04	0.05	6.9	7.1	1.0	1.0

Table (3) : Effect on some physical chemical characterizing of the fruits of Barhy date during 2016 & 2017 season .

Treatment	**Fruit weight (g.)**		**Fruit height (cm.)**		**Fruit diameter**		**Seeds %**		**Palp %**		**T.S.S %**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Control	14.1	13.8	3.59	3.58	2.45	2.48	7.0	7.1	93.0	92.0	24.0	23.9
Normal-B at 0.025 %	15.0	14.9	3.64	3.64	2.49	2.51	6.7	6.8	93.3	93.2	24.5	24.4
Normal-B at 0.05 %	15.9	16.0	3.68	3.70	2.54	2.55	6.5	6.6	93.5	93.4	25.0	25.1
Normal-B at 0.1 %	16.9	16.8	3.73	3.75	2.58	2.59	6.3	6.4	93.7	93.6	25.6	25.7
Normal-B at 0.2 %	17.0	17.1	3.74	3.76	2.59	2.60	6.3	6.3	93.7	93.7	25.6	25.7
Nano – Bat 0.0125 %	18.0	17.9	3.80	3.82	2.63	2.63	6.1	6.1	93.9	93.9	26.3	26.5
Nano – Bat 0.025 %	19.0	18.9	3.86	3.87	2.67	2.67	5.9	5.9	94.1	84.1	27.0	27.1
Nano – Bat 0.05 %	19.8	19.0	3.87	3.88	2.68	2.68	5.8	5.8	94.2	94.2	27.1	27.2
Nano – Bat 0.1 %	19.9	19.0	3.87	3.89	2.69	2.69	5.8	5.8	94.2	94.2	27.2	27.2
New 1- S. Dat 5 %	0.8	0.7	0.04	0.05	0.03	0.03	0.2	0.2	0.2	0.2	0.5	0.4

Table (4) : Effect on some chemical characteristics of the fruit of Barhy date palms during 2016 & 2017 season .

Treatment	Total sugars %		Reducing sugers %		Non-Reducing sugars %		Total acidity %		Total Fibre %		Total solble tannius %	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Control	22.0	22.2	18.1	18.0	3.9	4.2	0.391	0.385	0.89	0.90	0.87	0.85
Normal-B at 0.025 %	22.5	22.6	18.5	18.6	4.0	4.0	0.361	0.358	0.84	0.86	0.84	0.81
Normal-B at 0.05 %	23.0	23.1	19.0	19.0	4.0	14.1	0.330	0.338	0.80	0.81	0.79	0.78
Normal-B at 0.1 %	23.5	23.7	19.4	19.5	4.1	4.2	0.300	0.306	0.73	0.77	0.74	0.75
Normal-B at 0.2 %	23.6	23.8	19.5	19.6	4.1	4.3	0.299	0.301	0.72	0.76	0.73	0.74
Nano – Bat 0.0125 %	24.1	24.4	19.9	20.2	4.2	4.2	0.261	0.275	0.67	0.68	0.69	0.67
Nano – Bat 0.025 %	24.8	24.8	20.4	20.7	4.4	4.1	0.231	0.250	0.60	0.64	0.65	0.64
Nano – Bat 0.05 %	24.9	25.0	20.5	20.8	4. 4	4.2	0.230	0.249	0.59	0.63	0.64	0.63
Nano – Bat 0.1 %	25.0	25.1	20.6	20.9	4.4	4.2	0.229	0.248	0.25	0.62	0.63	0.62
New 1-S. Dat 5 %	0.4	0.4	0.2	0.3	NS	NS	0.025	0.024	0.03	0.03	0.02	0.02

Effect of Mycorrhizae inoculation on the growth of young date palm plants under nursery conditions

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Abstract

Two years' experiments were carried out at Al Hamrania Research Station (UAE) during (2015-2016) to explore the role of Mycorrhizal inoculation on the growth of date palm plants from micro-propagation growth under nursery conditions. Uniform plants of four date palm cultivars (Barhi, Khalas, Sultana and Madjool) were planted into 10-L pots. The Randomized Complete Block Design (RCBD) was used in this study experiments which involved application of: (T1) recommended 100% chemical fertilizer only, (T2) 100% of recommended organic fertilizer only, (T3) Mycorrhizae without any fertilization, (T4) Mycorrhizae + 100% recommended chemical fertilizer, (T5) Mycorrhizae + recommended 50% organic fertilizer, (T6) Mycorrhizae + 50% chemical fertilizer and (T7) Mycorrhizae +25% chemical fertilizers +25% organic fertilizers.

The results indicated that the use of Mycorrhizae with 100% of recommended organic fertilizers rate were increased significantly by 23% and 25% of the seedling leave growth rate and leave numbers per seedlings, respectively, when compared with the control treatment (without Mycorrhizae inoculation). This greenhouse study also indicated that half the amount of organic fertilizers application had similar effects when compared with 100% organic fertilizer when mixed with mycorrhiza for number of product leaves for all date palm cultivars.

Keywords: Mycorrhizae, Date palm, organic fertilizers, Compost, seedling, leave growth rate.

INTRODUCTION

Date palm is important crops in the United Arab Emirates, where numbers of date palm trees planted is increasing annually. Date palm trees are grown under harsh climatic conditions characterized by low rainfall and high evaporation rates and mainly with a weak soil material organic fertility.

Growing of date palm is accompanying with use of large amounts of organic and chemical fertilizers with high water supply. These practices may lead to salinization of soil and fertilizer salts to leak of deep soil layers which bad impacts on groundwater in addition to the high cost of fertilizers. Since most soils of United Arab Emirates are sandy and light texture, this limit their ability to retain water and fertilizer originally created in clay content and repotting and organic matter and also poor fertilizer formulations elements.

Most farmers in the United Arab Emirates to fertilize date palm trees by sprinkling the compost and organic fertilizer around the tree with global irrigation, but this method may not live up to expectations of higher growth and production because most fertilizers lose by washed away from the root zone or loss of soil surface directly.

Therefore, the use of advanced agricultural suitable techniques may become important in cultivating a sustainable date palm under the environmental conditions prevailing in the region to limit the negative effects of higher use of fertilizer and water scarcity and increased tree growth and productivity of promising technologies. That has drawn worldwide attention in recent years using Mycorrhizae which provides benefits through improvement of use of water and fertilizer by developing the ability of plants to absorb nutrients from the soil and increase their ability to withstand environmental stress factors Such as drought, salinity, etc.

Mycorrhizae can provide benefits to plants through the enormous plant roots volume that stretches into the soil to reach the soil layers away to common rooting zone to absorb minerals and transferred to the plant in addition to protecting the plant from soil pests such Nematodes, and improve soil properties and protected from erosion.

The present paper presents the results of use *Mycorrhizae* with young date palm plants produced by micro-propagation before their final plantation in the field.

Objectives of the study the sustainability and further development of palm cultivation and improve productivity study of saving in irrigation water and fertilizer amounts. additive and protect the environment from pollution and studying the efficiency of fertilizers on the date palm plants. This experiment serves also as filed study for Ministry staff and farmers in employment optimization of natural resources under the prevailing conditions.

MATERIAL AND METHODS

This study was conducted is conducted in Al Hamrania research station in Ras Al Khaima during three years (2015-2017). Plants used are “Khalas”, “Sultana”, “Barhi” and “Medjool” date palm cultivars of one year of age cultivated by micro-propagation. All the four cultivars are grown in Al Hamrania date palm gene bank with other more than 100 date palm accessions.

For all treatments when the *Mycorrhizae* was inoculated to plants with fertilizer. The quantity even organic and chemical fertilizer was 100 % of recommended quantity, at 50% and 25% quantity compared with *Mycorrhizae* alone. All treatments with control was seven in total.

All treatments received 25% of the water requirements based on Date palm in the field K_C irrigation by Allen et al. (1998). *Mycorrhizae* is added 50 cm depths around plant roots before applying fertilizer.

The Randomized Complete Block Design (RCBD) was used in this study experiments which involved application of 7 treatments using *Mycorrhizae* with organic and chemical fertilizers as recommended by the Ministry and applied to the young date palm plants when cultivated directly in filed. The composition shown in Table 1 is 100%, 59% and 25% of fertilizer (Organic and chemical) alone or mixed with or without inoculation by *Mycorrhizae*. As control *Mycorrhizae* was inoculated to young plants without any fertilization other than soil content.

RESULTS AND DISCUSSION

The results (Figure 1 and 2) indicated that the use of Mycorrhizae with 100% of recommended organic fertilizers rate were increased significantly by 23% of the plants number of leaves and by 25% the growth rate (leaves length after 22 months of growing under nursery conditions. It was related by Shabbir et al. (2011) that inoculation of date palm by Mycorrhizae initiate vegetal growth as number of leaves, their length and width and the trunk growth.

When compared with the control treatment (without Mycorrhizae inoculation). This study also indicated that half the supply rate (50%) of organic fertilizers application had similar effects when compared with 100% organic fertilizer in the treatment of inoculation of the young plants

by Mycorrhizae for the leaves numbers for all date palm cultivars. Young date palm plants growth quickly with inoculation by *Mycorrhizae* (Janos, 2007; Meddich et al., 2015).
It is recommended to continue following the plants under field conditions. The shape of plants need to moved them from nursery to plantation in the field.

It also recommended to reduce the supply of fertilizer (Organic or chemical) as no significate difference was recorded between 100% and 50% fertilizer supply. This confirmed other results that Mycorrhizae improve the roots absorption and use of water and fertilizers (Bouhired et al. 1992: Ghazi et al. 2013).

REFERENCES

Allen R.G., Pereira L.S., Raes D., and Smith M. 1998. Crop Evapotranspiration guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. FAO, Rome. 1998; 300(9): D05109

Bouhired, L., S. Gianinazzi and V. Gianinazzi-Pearson. 1992. Influence of endomycorrhizal inoculation on the growth of Phoenix dactylifera. In: Micropropagation, root regeneration and mycorrhizas. Joint meeting Plant Nutr. 46:291-297.

Ghazi N. Al-Karaki. 2013. Application of mycorrhizae in sustainable date palm cultivation. Emir. J. Food Agric. 2013. 25(11): 854-862doi: 10.9755/ejfa. v25i11.16499

Janos, D.P. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza 17:75–91.

Meddich A., F. Jaiti, W. Bourzik, A. El Asli and M. Hafidi. 2015. Use of mycorrhizal fungi as a strategy for improving the drought tolerance in date palm (Phoenix dactylifera). Scientia Horticulturae. Volume 192, 31 August 2015, Pages 468-474.

Shabbir, G., A. J. Dakheel, G. Brown, M. C. Rillig. 2011. Potential of arbuscular mycorrhizal technology in date palm production. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 449-476. Date Palm Biotechnology, Springer, Dordrecht.

Table

Table 1. Treatments applied on date palms plants of 1-year age from micro-propagation

Treatment	Composition
T_1	Chemical fertilizer alone (100%)
T_2	Organic Fertilizer alone (100%)
T_3	Mycorrhizae alone
T_4	Mycorrhizae + 50% of Chemical fertilizer
T_5	Mycorrhizae + 50% of Organic Fertilizer
T_6	Mycorrhizae + 100 % Organic Fertilizer
T_7	Mycorrhizae + Chemical fertilizer 25%+ Organic Fertilizer 24%

Figures

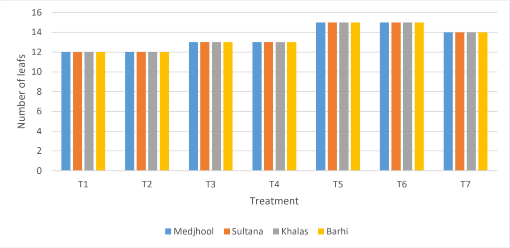

Fig. 1. Length of date palm leaves of 4 cultivars of date palm inoculated and no inoculated by *Mychorrizae* after 2 years' cultivation under nursery

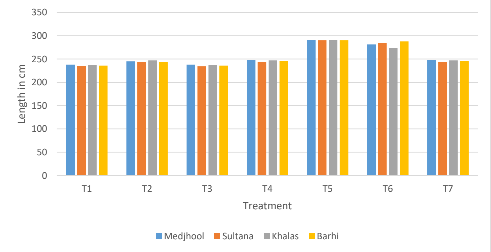

Fig. 2. Number of date palm leaves of 4 cultivars of date palm inoculated and no inoculated by *Mychorrizae* after 2 years' cultivation under nursery

Plants in April 2015 (starting experiment)	
Plants in July 2015 (after 3 months of inoculation)	
Plants in January 2016 (after 9 months of inoculation)	
Plants in February 2017 (after 24 months)	

Fig. 3. Evolution of the date palm plants growth under nursery conditions in Al Hamrania Research Station-UAE (2005-2007).

Sustainable Irrigation Management with Saline Groundwater of Three Date Palm Cultivars in the Hyper-Arid United Arab Emirates

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Abstract

The United Arab Emirates annually produces nearly 1 million tonnes of dates, which is nearly 12% of the world's production. Date palms (*Phoenix dactylifera* L.) cover nearly 200,000 hectares. Irrigation is essential. Groundwater is used for irrigation, and date palms account for one third of the water allocated for irrigation. However, 64% of the groundwater extracted is drawn from aquifers in excess of their renewal rates. Furthermore, the groundwater supplies are becoming more saline and date palms are sensitive to salinity. Sustainable irrigation practices need to be developed.

INTRODUCTION

In 2014, a pilot experiment was set-up at the International Center for Biosaline Agriculture (ICBA) near Dubai. The focus was to determine the water use of the cultivar 'Lulu' being irrigated twice daily with 5 dS/m water (Fig. 1). Tree water-use was measured directly using sap-flow sensors placed in the tree trunks, and indirectly using time domain reflectometry in the root-zone.

Local weather data were used to calculate the hourly and daily ETo, and derive an appropriate value for the crop factor, Kc. Our data showed the water use of the palm trees to be less than half the amount suggested by the FAO-56 guidelines. Furthermore, much of the irrigation water was seen to be rapidly lost by deep drainage through the highly permeable sands.

In 2015 a comprehensive project commenced on two additional cultivars ('Shahlah' and 'Khalas'). The experiments were extended for all cultivars to irrigation with 15 dS/m water. We have measured a decline in tree transpiration in relation to the irrigation water salinity, and for the cultivar 'Lulu', the increase in salinity from 5 to 15 dS/m results in a halving of the tree's water use (Fig. 2).

Part of this drop is due to the impact that salinity has had on the leaf area of the canopy. To measure the leaf area of the palm trees' canopies we have developed a light stick that measures the area of the shadow cast by the trees (Fig. 3).

Our measurements show that the salinity rise has reduced the 'Lulu' canopy area by only 25% (Fig. 4). Other processes must be involved, and these likely involve stomatal control of transpiration as affected by salinity. We will investigate this.

CONCLUSIONS

- Irrigation of date palms consumes one third of the UAE's groundwater take, and this resource is dwindling and becoming more saline.
- We have shown that irrigation can be reduced to better match the palm's needs for water and yet maintain a leaching fraction to flush salts away.
- Tree water use is reduced when higher salinity irrigation water is used.
- The reduction in water use is due not only to the reduction in the tree canopy area with salinity, but also due to physiological processes.
- A decision support tool is being developed to optimize irrigation use.

Figures

Fig. 1. One of the instrumented 'Lulu' date palm tree in the low salinity (5 dS/m) treatment at the International Center for Biosaline Agriculture. The irrigation bubbler is in operation.

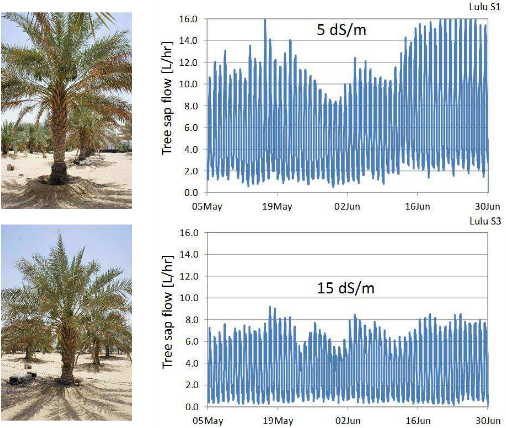

Fig. 2. Comparison of the measured pattern water use of the 'Lulu' date palm trees irrigated with 5 dS/m water (S1, top) and 15 dS/m water (S3, bottom). The measurements were made every 30 min throughout the day.

Fig. 3. Ahmed Al Muaini measuring the area of the shadow cast by date palm trees using the light stick. The inset show the details of the light sensors. There are 20 sensors on the stick.

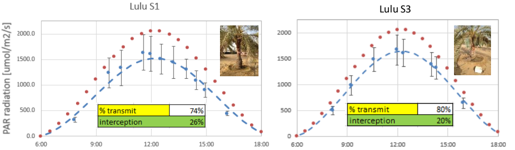

Fig. 4. Measurements of light interception by the canopy of the 'Lulu' trees in the low salinity treatment (S1, 5 dS/m, left) and the high salinity treatment (S3, 15 dS/m, right).

General Topic on Date Palm

Physio-chemical, flavor compounds and sensory properties of some UAE commercial date syrups

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Abstract

Date palm (*Phoenix dactylifera L.*) is the major fruit produced in the United Arab Emirates (UAE). Date is consumed as fresh fruit at different ripening stages. Low quality date is processed to date syrup (Debis) and date paste. Date syrup is produced commercially by extracting and concentrating the simple sugars and other water soluble compounds. Debis is consumed directly or used as sweetener, colorant and flavoring ingredient in different food products. The aim of the study was to investigate the physical (color, viscosity), chemical (proximate composition, sugars and flavor compounds) and sensory properties of some commercial date syrups available at UAE market.

The evaluated date syrups had similar proximate composition but differ on color, sugar, viscosity and sensory properties. One hundred sixty-seven flavor compounds were identified mainly alcohols, esters, aldehydes and ketones. The type, number and amount of the flavor compounds were different on each date syrup. Alcohols percentage was the highest in all date syrup brands followed by esters in some syrups and ketones in the other syrups. Each date syrup had different dominant flavor compounds. Date syrup quality depends on the type of date used. The commercial date syrups were extracted from mixed date varieties not mentioned on the product labels. Results of the study provided the first comprehensive flavor profiles and aromatic volatiles of some commercial Emirati date syrups.

Keywords: Date fruit syrup, sugars, sensory properties, flavor/aroma volatile.

1. INTRODUCTION

Date palm (***Phoenix dactylifera)*** is among the most important crops grown in the Middle East, and Arabian Peninsula (Aslam et al., 2013). Its fruit is sweet berry holds digestible sugars (70%), mostly glucose, sucrose, and fructose; dietary fibers (6.4-11.5%) and contains less proteins (2.3-5.6%) and fat (0.2-0.5%) (Al-Farsi *et al*., 2007; Al-Shahib & Marshall, 2003).

The date is usually graded, sorted, and commercially packed by the producer and a significant portion of dates that are undersized, damaged and unattractive are wasted in such process; making a real economic loss as they are rich in bioactive compounds that can be used as value added materials (Besbes et al., 2009, Elleuch et al., 2008**).** Date processing industries made a boost in economy by utilizing wasted dates and manufacture variety of date products like date-paste and date syrup and other products (Ahmad *et al*., 2013).

Date syrup known as (dibs) is the most commonly derived date product (El-Nagga and El-Tawab, 2012), it is produced by mixing pitted date fruits with water and heating them when sugars are extracted. This method ensures the destruction of some nutritive components and it darkens the color of the product (Al-Farsi *et al.,* 2005). Dibs can be used as a functional food ingredient since it is a good source of reducing sugars, minerals and total phenolics, and it can replace sucrose in food products (El-Nagga & El-Tawab, 2005, Al-Sharnouby *et al.,* 2014)

Fruit flavor as well as color and texture controls fruit quality, it depends on the both the production of volatile compounds (aroma) and the perception in mouth (sweetness, acidity, or bitterness) (Ogundiwin *et al.,* 2009, Maffei, 2010). Not enough data is available regarding Emirati date syrup, consequently, the aim of this research, is to evaluate the quality of different commercial date syrup types in the market of the United Arab Emirates, by analyzing their aroma volatiles and sugar composition.

2. MATERIALS AND METHODS

2.1 *Materials*

Eight syrup commercial brands collected from the local markets of the United Arab Emirates. Date syrup was enclosed in a plastic jar, and stored at room temperature in a dark place.

2.2 *Proximate analysis*

Date syrup samples were analyzed for moisture content using Karl Fischer method, protein by Kjeldahl nitrogen (method 920.152), and ash by oven were determined according to the Association of Official Analytical Chemists' method (AoAC, 1995). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25 (AOAC, 1995). The Bligh and Dyer method (Hanson & Olley, 1963) was used to determine the lipid content. Total carbohydrates were calculated by subtracting the total percent values of other measurement from 100.

2.3 *Texture Analyzer*

For two-cycle compression, a Texture Analyzer Hybrid rheometer was used to measure the force time curve. All measurements were carried out in a controlled room temperature at 25 °C. The 25kg load cell was calibrated with a 5kg weight. Cubical samples (3 cm length) were prepared from date paste pack for texture analysis and placed carefully on the metallic surface of the Texture Analyzer. Then the cross head was allowed to penetrate at the rate of 0.5 mm/s to a total deformation 5.0 mm and back to original position followed by second down and up cycle on the same sample. All operations were automatically controlled by the TexureProan excel based software attached with the instrument. Texture parameters were calculated from the software. The instrument automatically recorded the force displacement or forcetime curve. Three replicates were conducted for each cultivar (Ahmad and Ramaswamy, 2006).

2.4 *Viscosity*

The viscosity of samples was measured at 25°C, using the "Discovery HR-2 hybrid rheometer". The stainless-steel rotor (36.8 mm outside diameter, 60 mm length) was used, this provided an annular gap of 2.6 mm between the rotor and the walls of the sample beaker. The sample ware placed in small cups weighted 10 g.

2.5 *Color*

Visual shade was measured utilizing a Hunter colorimeter as far as L (lightness), a (+ redness and - greenness) and b (+ yellowness and -blueness).

2.6 *Total soluble solids (TSS)*

For the total soluble solids, Abbe Refractometer was used for date syrup samples. 0.5g of the sample was placed in the measuring plate.

2.7 *Sensory evaluation*

Sensory profile was conducted using ten trained panelists. The panel was trained to establish date flavor attributes and agreed on the date reference ratings. Sensory quality was determined using quantitative descriptive analysis test. Date syrups were presented individually and in random order, and then assessed by the trained panel using 15 cm unstructured line scales with the anchor points low intensity and high intensity on the left and right sides of the scale. The sensory profile included 18 descriptive attributes (8 aromas, 2 basic tastes and 8 flavors). Date syrups were presented in small plastic cups labeled with three-digit random number. Panelists were instructed to take three short sniffs to evaluate the aroma attributes, evaluate the taste and flavor attributes and drink water before evaluating the next sample. Sensory tests were conducted in a sensory laboratory with separate booths under similar temperature and light conditions. The intensity determination of the sensory perception was replicated three times.

2.8 *Sugar extraction*

Glucose, fructose, and sucrose were determined using High Performance Liquid Chromatography (HPLC). Date syrup samples were homogenized and mixed with a mixture of acetonitrile and water (50:50), and then stirred continuously for 15 minutes on a magnetic stirrer, followed by a centrifugation for 10 min at 10,000 rpm. 5ml of the supernatant was then passed through a 0.22 μm pore size organic syringe filter (30 mm diameter (Millipore)), and 5μl of the filtrate was injected for sugars analysis. The extract was analyzed using HPLC system (Ultimate 3000, Dionex, Germany), equipped with an auto sampler. The injection volume was 5 μL and the detector used was an Ultimate 3000 Variable Wavelength detector at 190 nm. Carbohydrate column used was U-Bondapack-NH2 (300 mm length x 3.9-mm id x 10 um particle size, Waters 084040). The mobile phase used was a mixture of acetonitrile and type I water (83:17) with an isocratic flow rate of 1.5 mL min^{-1} at a temperature of 35°C. The peaks were compared with peaks from food sugar standard (fructose, glucose, sucrose, Sigma-Aldrich; CAR-11) which was prepared at 20 mg/mL in 70/30 ACN/H2O. Other standards were prepared in 50/50 ACN/H2O at concentrations from 2mg/ml to 10 mg/ml. UV absorption used for detection was near the 190 to 195 nm, Consequently, type I water and Acetonitrile; UV cutoff, 190 nm are used as the mobile phase and the sample is relatively uncontaminated.

2.9 *Volatile Extraction*

Weighing approximately 0.500 gram from each sample with 1 ml of distilled water in a pyrex rubber cap tube and letting them getting homogenized with the help of a magnetic stirrer for 30 minutes at (350 rpm). Then, a Tenax from GERSTEL company was connected to a vacuum pump at its upper end, the other (lower) end of it was inserted to the sample-containing tube for another 30 minutes before injecting both the Tenax and the tube on GC-MS. The analyses were performed using a 3800 GC equipped with a 4000 Mass Selective Detector (Varian), Thermal desorption Unit (TDU, Gerstel), PTV inlet (CIS 4, Gerstel) and MPS 2 with headspace and DHS (Gerstel). Analysis conditions: PTV: Tenax TA liner, solvent vent (60 mL/min) at 0 kPa splitless (2 min), 20°C (0.2 min); 10°C/s; 300°C (5 min), Column: 25 m CP-SIL 5CB (Varian), di = 0.15 mm df = 2.0 μm, Pneumatics: He, constant fl ow = 0.5 mL/min, Oven: 40°C (10 min); 10°C/min; 300°C (6 min), MSD: Scan, 28 - 350 amu.

Constituents were identified based on a comparison of the retention times with those of authentic samples, comparing their relative retention indices to an online library mass spectra literature data (flavornet.org)

2.10 *Statistical Analysis*

Statistical analysis was carried out using SPSS 18. Trends were considered significant, while means of compared sets differed at $P<0.05$.

3. RSULTS AND DISCUSSION

3.1 Proximate Analysis

Table 1 Proximate analysis of date syrups

Syrup sample	Moisture	Ash	Lipid	Protein	Carbohydrates
1	20.4 ± 0.3^{a}	1.7 ± 0^{a}	0.8 ± 0.0^{b}	3.1 ± 0.6^{a}	73.8
2	22.0 ± 1.2^{ab}	1.7 ± 0^{a}	0.6 ± 0.1^{ab}	2.2 ± 0.3^{a}	73.2
3	21.8 ± 0.7^{ab}	1.8 ± 0^{a}	0.3 ± 0.0^{a}	2.4 ± 0.3^{a}	73.5
4	25.3 ± 1^{c}	1.3 ± 0^{a}	1.4 ± 0.1^{c}	2.0 ± 0.3^{a}	69.8
5	23.0 ± 1.4^{bc}	1.4 ± 0^{a}	0.6 ± 0.0^{ab}	2.5 ± 0.4^{a}	72.3

Means with different letters are significantly different according to Duncan's test

From table 1, it was well observed that carbohydrate was the predominant component in all date syrup samples, followed by moisture, along with small amounts of protein, fat, and ash. Moisture contents of date syrup varied between 20.4 g/100 and 25.3 g/100 g, being lowest in sample (1) and recorded the highest in sample (4), whereas lipids were not high, ranged from 1.4 g/100 g in sample (4) to 0.3 g/100 g in sample (3). Protein ranged between 3.1 g/100 in sample (1) and 2.0 g/100 in sample (4). Ash was the highest in sample (3) 1.8 g/100 and the lowest in sample (4) 1.3 g/100. Ash and protein in all samples were not significantly different.

Product	Viscosity Pa. s
1	**34.64 ± 1.07^{e}**
2	**0.82 ± 0.56^{a}**
3	**7.98 ± 1.0^{c}**
4	**4.44 ± 0.90^{b}**
5	**11.12± 0.28^{d}**

3.2 Color

3.3 Total Soluble Solids (TSS)

3.4 Viscosity

Viscosity of date syrups was investigated at temperature 25 °C. Sample (1) possessed the highest viscosity and sample (2) was the lowest. There was a significant difference between all samples

3.5 Sensory Evaluation

The students from the sensory evaluation laboratory measured 9 parameters, color, glossiness, aroma, viscosity, thickness, sweetness, smoothness, flavor and overall quality of date syrup samples. From table 3, there was a significant difference in color between samples (1), (2) and (3), and a significant difference in glossiness between samples (1) and (2), but in aroma, there was no significant difference between samples. Viscosity in samples (1), (2) and (3) was significantly different, while thickness was significantly different in samples (3), (4) and (5). Significant difference was detected between samples (1) and (2) in terms of sweetness, smoothness, and flavor.

Table 2. Visual shade of date syrup samples

Syrup sample	Lightness	a (+ Redness & - greenness)	b (+ yellowness & - blueness)
1	9.45 ± 0.085^{d}	6.26 ± 0.46^{c}	10.55 ± 0.53^{c}
2	5.89 ± 0.09^{a}	3.81 ± 0.19^{a}	5.48 ± 0.18^{a}
3	8.80 ± 0.089^{c}	5.04 ± 0.13^{bc}	9.30 ± 0.06^{b}
4	5.81 ± 0.22^{a}	4.65 ± 0.20^{ab}	6.26 ± 0.04^{a}
5	8.09 ± 0.07^{b}	3.56 ± 0.40^{a}	8.67 ± 0.05^{b}
Means with different letters are significantly different according to Duncan's test (P>0.05)			

3.6 Sugar Composition

The content of glucose and fructose of seven date palm syrup samples is shown in table 4, the percentage of fructose is higher than the percentage of glucose in all brands, AlRimal and AlKharj brands had the highest sugar percentage, followed by Safa and Marbea AlGebria, and Date crown, Azzad, and Alhijaz were the lowest.

The variety of date palm fruit and the level of maturity affect the chemical composition of date palm fruit (Mostafazadeh *et al.,* 2011, Ahmad *et al*., 1995, Tang *et al*., 2012, Al-Farsi et al.,2007; Ismail et al., 2006; El Arem et al., 2011; Nasir et al., 2015). The possible use of several date fruit varieties at different ripening stages making the syrup, would possibly explain the variation of sugar composition between syrup brands. However, the conversion process of dates to syrup in the industrial factories and the storage conditions would play a crucial role in the stability of sugars originally found in date fruit.

Table 4. Percentage of glucose (Glu), and fructose (Fru), of seven Emirati date palm fruit syrup

	1	2	3	4	5	6	7
% Glu	37.43	37.81	40.69	37.25	41.29	38.125	38.325
% Fru	41.725	38.845	41.115		43.045	38.4	34.26

Table 3
Sensory characteristics of commercial date syrup samples (n=22)

Sample	Color	Glossiness	Aroma	Viscosity	Thickness	Sweetness	Smoothness
1	12.6±0.5^{a}	10.1±0.7^{a}	8.5±0.5^{a}	11.9±0.4^{a}	9.9±0.7^{a}	9.7±0.5^{a}	4.0±0.6^{a}
2	11.4±0.4^{ab}	11.9±0.4^{ab}	8.5±0.6^{a}	8.6±0.7^{ab}	7.0±0.6^{a}	8.2±0.9^{ab}	12.1±0.5^{b}
3	9.9±0.5^{bc}	11.7±0.4^{ab}	10.1±0.5^{a}	7.2±0.6^{bc}	6.6±0.5^{ab}	11.5±0.3^{c}	12.7±0.4^{c}
4	9.1±0.6^{c}	11.2±0.5^{ab}	10.2±0.5^{a}	10.3±0.6^{c}	8.2±0.6^{bc}	11.5±0.4^{c}	12.0±0.5^{c}
5	12±0.3^{c}	10.0±0.6^{b}	10.1±0.4^{a}	11.2±0.5^{c}	10.3±0.5^{c}	11.2±0.3^{c}	7.0±0.8^{c}

*abc of the same column bearing different superscripts differ significantly from each other in terms of D

3.7 Aroma Volatile Identification

More than 50% of total aroma was detected from each date fruit syrup brand, 212 volatile compounds were identified (Table 5); 65 alcohols, 30 aldehydes, 42 esters, 42 ketones, 6 carboxylic acid, 14 saturated hydrocarbons, and 26 unsaturated hydrocarbons. Figure **1** shows the percentages of each chemical group in date palm syrup brands, it is obvious that the percentage of alcohols, ketones and esters in most date syrup brands were the highest.

In Golden Date syrup, 84.4% of aroma was identified, 47.7 % of total aroma was alcohols, and the main volatile compound was pentanol (30%), and the other main volatile compound was pentanone (10.8%). The percentage of aroma identified in Date Crown was less the aroma of Golden Date (62.2%), alcohols and esters were the most abundant volatile groups, account for 19.6% and 14.2% respectively of total aroma, and the compounds ethyl hexadecanoate, isopropyl palmitate, and dimethyl disulfide were the main volatiles. There was a similarity between Azzad and AlRimal date syrup brands brands; the main volatile groups responsible for the aroma were alcohols and ketones, followed by aldehydes, esters and unsaturated hydrocarbons, but the percentage of carboxylic acid in Azzad it was the highest among other brands (3%).

Table 5
Composition of volatile compounds obtained from eight Emirati commercial date palm fruit syrup

Volatiles	DB-5	1	2	3	4	5	6	7	8
Alcohols									
propanol	536					0.4		2.52	0.47
methylbutenol	620	1.77							
isobutanol	647		1.07						
pentanol	759	30	2.39					4.23	
methylpentanol	768						0.29		
methyl-2-butenol	779							21.8	
ethoxypropanol	833	2.54	0.12			0.63		1.33	
furfuryl alcohol	851								1.55
dimethylthiazole	878						0.09		
fufuryl mercaptan	912							0.35	
heptanol	925			0.22					
dimethylthiazole	928							0.45	0.46
2-octanol	984						0.3		
2,3-dehydro-1,8-cineole	992					0.14			
trimethylthiazole	995			0.26	0.41		0.38	0.16	
1,4-cineole	1018					0.1			
(E)-3-hexenol	1038					0.53			
6(10)-dihydromyrcenol	1053					0.22			
p, a -dimethylstyrol	1069							0.9	
octanol	1072						0.3		
guaiacol	1089					0.02			
(E)-p-mentha-2,8-dien-1-ol	1122		0.19	0.32	0.47			0.99	
pinenol	1123					0.04	0.39		0.16
β-terpineol	1144		0.12						
p-cymenol	1166		0.08						
nonadienol	1167						0.07		
(E)-linalool oxide	1172								0.16
phenylethylthiol	1176					0.2		0.16	
neoisomenthol	[1199]			0.12				0.47	

(E)-carveol	1217			0.15					
(Z)-piperitol	[1220]						0.06		
benzothiazole	1240	0.99							
p-anisyl alcohol	[1258]	0.32							
p-menthenethiol	1283	1.01							
4-ethylguaiacol	1287			1.49					
indole	1292	0.34							
cinnamyl alcohol	1312	1.33		0.85					
undecanol	[1371]						0.06		
cis-linalool pyran oxide	[1402]		0.12			1.39	0.19	0.86	
decyl alcohol	1408	0.14							
geosmin	1412					0.19			0.26
isogeraniol	[1464]					0.66	0.39		
elemol	1547		1.67	3.79	5.64	1.76		2.62	8.39
lauryl alcohol	1577							0.09	
dill apiol	1602						0.38		
cedrenol	1604	2.35							
levomenol	[1620]			2.21					3.34
β-caryophyllene alcohol	[1642]		1.45	0.46				0.34	0.81
α-bisabolol	1662		0.83		2.16	0.88	1.66	1.35	3.28
zingiberenol	[1689]						0.2		
(E)-2-dodecen-1-ol	[1692]			0.39					
viridiflorol	[1754]	0.43				0.03		0.13	0.07
methyl furaneol	[1763]		0.95	1.12	5.55	0.86	0.79		3.7
uncineol	[1837]		1.55			0.23			0.14
hexadecanol	1870			0.47			0.2		
eudesmol	[1896]			0.87					
syringol	[1948]								0.29
(E)-isoeugenol	[2024]	2.93	2.5	1.16		0.16			
4-vinylphenol	[2079]		2.74	7.31	13.7	2.17	1.64	2.65	3.44
tetradecanol	[2116]	1.84	0.83	1.34					1.02
phytol	[2128]	0.66		1.54		0.11	1.11	1.06	0.39
5-oxymethylfurfurole	[2164]	0.82	2.48	4.84		0.08			0.5
6-Methoxyeugenol	[2222]	0.2				0.2			0.23
stearyl alcohol	[2232]		0.49	0.38	0.54	0.08		0.39	
3-oxo-α-ionol	[2302]					0.11	0.29		
% Identified		**47.7**	**19.6**	**29.3**	**28.5**	**11.2**	**8.78**	**42.8**	**28.7**
Aldehydes									
propanal	506		2.01						
methylbutanal	641			0.49		5.39	2.04		
pentanal	732	0.26		2.15		1.5			
diethyl acetal	734							1.04	1.15
methyl-2-butenal	[753]	6.2							
(E)-2-hexenal	[844]	0.97				1.47			
2-hexenal	854	0.52					0.97		
3-hexenal	800					6.83			
4-heptenal	902								0.54
methional	909					0.22			
2-hepten-1-al	951	0.65						0.15	
heptenal	957		0.37	0.55	0.66	0.32	0.98		
benzaldehyde	960								0.74
2-octenal	1060						0.18		
3-nonenal	1096			0.2					0.04
(Z)-4-decenal	1200				0.29	0.03	0.18		
decanal	1209						0.06		
2,4-nonadienal	1217							0.18	

2-decenal	1262			0.89					
p-anisaldehyde	1263		0.32			0.67	0.38	0.56	
perilla aldehyde	1271	0.31	0.27	0.54			0.22	0.41	
undecanaldehyde	1291						0.69		
decadienal	1297				0.95	0.34	0.33		0.63
decadienal	1317		0.73		1.81	0.74	0.79	1.35	1.36
(E)-2-undecenal	1366						0.06	0.09	
vanillin	1410		0.16	0.13	0.37				
2-dodecenal	1462			0.32	0.72			0.78	3.04
β-sinensal	1706			0.58				0.62	1.27
pentadecanal	[1711]		0.68		0.93		0.42	0.36	
palmitaldehyde	[1813]					0.19			0.09
octadecanaldehyde	[2052]	1.72	1.38		3.37		0.23		0.63
% Identified		**10.6**	**5.93**	**5.85**	**9.09**	**17.7**	**7.54**	**5.54**	**9.48**
Ketones									
methyl ethyl ketone	597							2.05	
pentanone	[636]	10.8				3.75			1.08
methylpentanone	[655]			2.12			2.52		
2,3-pentadione	700					3.23			
acetoin	718			0.36					
4-methyl-3-penten-2-one	798								9.81
hydroxypentanone	803			4.58					
methylbutanone	[866]		0.13	0.7		1.33	0.45	1.41	1.23
heptanone	895			0.14				0.4	
1-cyclohexen-3-one	914						0.09		
acetylpyrroline	923	0.16						0.27	
mercaptomethylpentanone	944						0.61		0.64
methyldihydrothiophenone	998								0.54
3-octen-2-one	1040		0.39						
acetophenone	1041				1.02				
4-mercapto-4-methyl-2-pentanone	[1043]					0.53			0.46
artemisia ketone	1062		0.26						
3,5-octadienone	1095						0.37	0.39	
DL-carvone	1253							0.49	
5-octanolide	1288		0.86		1.51	0.68		1.24	0.79
undecanone	1296		0.3	0.57				0.55	
ethylfuranone	[1342]					0.07	0.11		
(E)-β-damascone	1415						0.27		
α-ionone	1422	0.7							
wine lactone	1456	0.25							
γ-decalactone	1472		0.75			0.23	0.18	0.49	
β-ionone	1493	0.07							
raspberry ketone	[1515]				1.52		0.37	0.74	1.02
δ-undecalactone	1606			3.23					
epoxy-β-ionone	[1610]		1.2						
(E)-Whiskey lactone	[1629]							1.56	
(Z)-6-dodecen-γ-lactone	1656	1.56						0.21	
(Z)-6-dodecene-γ-lactone	1660			1.86					
pantolactone	[1685]							0.19	0.3
7-methoxycoumarin	1743					0.23	0.26	0.29	0.15
hexadecanone	[1798]						0.21		0.06
4-Carbethoxybutyrolactone	[1893]	0.23							
(+)-nootkatone	1814		4.12						
γ-undecalactone	[1922]		3.07	2.29	1.48		1.29	1.59	1.6
trimethylphenylbutenone	[1942]			0.66		0.18			
(E,E)-farnesylacetone	[2015]			6.4	6.72				

acetovanillone	[2292]	0.11	0.2			0.12			
% Identified		**13.9**	**11.3**	**22.9**	**12.3**	**10.3**	**6.73**	**11.9**	**17.7**
Esters									
butyl isothiocyanate	946						0.37		
methyl octanoate	1041						0.53	1.47	
ethyl formate	[547]							4.89	2.66
ethyl acetate	628		0.38						
ethyl propionate	713	1.65							
methyl butanoate	723						1.2	2.35	0.39
ethyl isobutyrate	756					1.59			
ethyl-(2R)-methyl-(3S) hydroxybutanoate	848			0.71				1.5	
methyl-2-(methylthio)acetate	894					0.77			
ethyl mercaptopropionate	[918]					0.39			
ethyl 3-hydroxybutanoate	935	0.09							
pentyl butanoate	[1094]					0.04			
(Z)-3-hexenyl butanoate	1178						0.06		
methyl salicylate	[1234]		0.33						
isobornyl formate	[1245]					0.17			
isopulegyl acetate	[1275]					0.18			
ethyl undecanoate	[1307]			0.2		0.08		0.23	
butyl benzoate	[1343]							0.1	
citronellyl acetate	1357	0.09	0.07	0.12	0.32	0.17	0.09	0.27	0.37
benzyl butanoate	[1335]					0.21		0.08	
hexyl hexanoate	1379		0.14						
geranyl acetate	1382	1.51					0.12		
linalyl butyrate	[1437]							0.07	
ethyl-(E,Z)-2,4-decadienoate	1479						0.13		
bornyl butyrate	[1490]								0.31
methyl laurate	1509	0.86					0.03		
geranyl butyrate	[1549]						2.25		
isopropyl benzoate	[1567]								0.21
geranyl isovalerate	[1610]				3.34	1.52	1.79	2.44	6.24
citronellyl valerate	[1625]						1.16		
benzyl benzoate	1723	0.98	1.33	2.69	2.71			1.56	
ethyl-(E)-cinnamate	[1749]		0.44						
bornyl benzoate	[1766]							0.59	
Diethyl 2-hydroxyglutarate	[1823]	0.82				0.11	0.23		0.08
phenylethyl benzoate	1841						0.29		0.08
p-hydroethylbenzene	[1847]				1.59	0.19	0.2	0.74	0.11
ethyl hexadecanoate	[1902]		4.29		0.32	0.1			
(E,E)-farnesyl acetate	[1935]			0.71					0.22
isopropyl palmitate	[2010]		5.4			0.14		1.51	1.71
methyl octadecenoate	[2082]		1.13						
methyl vanillate	[2250]	0.35	0.39	0.34		0.17			0.67
ethyl vanillate	[2264]	0.21	0.31	0.52	0.66		0.19	0.33	0.06
% Identified		**6.55**	**14.2**	**5.28**	**8.94**	**5.84**	**8.66**	**18.1**	**13.1**
Carboxylic Acids									
acetic acid	600					1.17			
pentanoic acid	911		0.19						
caproic acid	1019						0.57		0.44
nonanoic acid	1275				0.29				
decanoic acid	1373							0.32	
lauric acid	[2169]		0.63	3	0.89			0.94	
% Identified		**0**	**0.82**	**3**	**1.19**	**1.17**	**0.57**	**1.26**	**0.44**
Unsaturated hydrocarbons									

fucoserratene	[760]			4.37		3.04	1.97		
methylthiophene	765				3.49				3.75
o-picoline	[886]							0.23	
dimethyl pyrazine	892	0.29							
α-pinene	939	0.18		0.36		0.2			
methyl-p-xylene	[945]					0.24			
methylethylpyrazine	[1035]	0.55					0.21		0.1
(Z)-ocimene,	1043			0.39					
2-methoxy-3,6-dimethylpyrazine	1065			0.27					
ethyldimethylpyrazine	1083							0.18	0.04
acetylthiazoline	1105		0.13						
p-menthatriene	1115	0.17				0.29			
diethylmethylpyrazine	1160						0.16	0.39	
epoxy-p-menthene	1235						0.2		
p-mentha-dien-hydroperoxide	1327						0.05	0.15	
δ-elemene	1340			0.05					
α-gurjunene	1412							0.33	
(-)-γ-elemene	1425		0.48	1.01	1.31	0.52	0.53	0.96	1.55
germacrene D	1487		0.11		0.12	0.31	0.16	0.07	
(-)-β-bisabolene	1498		0.14			0.12	0.12	0.11	
elemicin	1514		0.45	1.09		0.97			
β-sesquiphellandrene	[1560]							0.3	
(E)-isoelemicin	1596	0.31							
2,6-dimethylnaphthalene	[1690]		0.51						
α-calacorene	[1859]						0.18		
hydroxycalamenene	[2085]		2.06						
% Identified		**1.49**	**3.88**	**7.55**	**4.92**	**5.68**	**3.57**	**2.73**	**5.44**
Saturated hydrocarbons									
dimethyl disulfide	785		5.52			6.6	16.7		
5-(methylthio)-valeronitrile	1015							0.17	
(+)-cis-rose oxide	[1109]						0.18		
nerol oxide	[1131]		0.06	0.12	0.33			0.4	
limonene oxide	[1132]						0.22		0.14
dimethyl tetrasulfide	1232							0.1	
erucin	1447							0.03	
bis(2-methyl-3-furyl)disulphide	1540	3.16							
hexadecane	1600			1.15	0.53	0.21		0.63	1.42
octadecane	1800				0.5	0.04		0.4	
eicosane	2000					0.29	0.59		
heneicosane	2100	0.87	0.94	0.87				1.04	0.76
docosane	2200	0.18				1.22	0.33	1.2	1.37
tricosane	2300			0.56				0.3	
% identified		**4.21**	**6.52**	**2.7**	**1.36**	**8.36**	**18**	**4.26**	**3.7**
% total identified		**84.4**	**62.2**	**76.6**	**66.2**	**60.3**	**53.8**	**86.6**	**78.5**
Number of identified compounds		**48**	**58**	**59**	**33**	**74**	**75**	**80**	**63**

Aldehydes were the main volatiles in AlHijaz date syrup, account for 17.7% of total aroma, and the most abundant compounds were methylbutanal and 3-hexenal, the percentage of alcohols was (11.2%), ketones (10.3%), and saturated hydrocarbons (8.36%), while esters, unsaturated hydrocarbons and carboxylic acids were found in way less amounts. AlKharj date syrup had the highest percentage of saturated hydrocarbons among other brand (18%) and the main compound was dimethyl disulfide, with 16.7% of total aroma, AlKharj date syrup also had the lowest aroma detection (53.8%). The aroma of Safa syurup had significantly large amounts of alcohols (42.8%), esters (18.1%), and ketones (11.9%), and methy-2-butenol was the main compound with 21.8% of total aroma. Arabic coffee date syrup had high percentage of alcohols, ketones, and esters (28.7%, 17.7%, and 13.1% respectively), where 4-methyl-pentenone was the most abundant volatile compound. The ester compound (citronellyl acetate) was the only volatile compound detected in all date syrup samples (Fig. 4).

It was observed that the number of volatile compounds identified in each date syrup samples vary; 80 volatiles identified in Safa date syrup,75, 74, 63 59, 58, and 48 were identified in AlKharj, AlHijaz, Arabic Coffee, Azzad, Date Crown and in Golden Date syrup brands respectively, and the least compound variation was observed in AlRimal date syrup, with 33 volatiles.

The differences in aroma profiles between all date syrup brands in terms of volatile composition and variation would be resulted from two possible suggestions; either from the level of maturity of dates and the type of date fruit the syrup made of, or by the effect of industrial treatments and backaging that led to loss of volatiles

4. CONCLUSION

The amounts of glucose and fructose and the aroma volatiles extracted from different commercially produced date fruit syrup brands are not similar, few factors may affect the characteristics of date fruit syrup; the origin of date fruits, the variety, the level of ripening, the post-harvest treatments and the environmental factors can be the main cause, in addition to impact of the process when transforming fresh dates into syrup product.

REFERENCES

Ahmed, Imad A., Ahmed, Abdul Wahab K. & Robinson, Richard K. (1995). Chemical composition of date varieties influenced by the stage of ripening. Food Chemistry 54:305-309

Ahmed, J. Hosahalli S. Ramaswamy. (2006). Physico-chemical properties of commercial date pastes (Phoenix dactylifera). Journal of Food Engineering 76:348–352

Ahmed, J. A. Almusallam, Al-Hooti, S. N. (2013). Isolation and characterization of insoluble date (Phoenix dactylifera L.) fibers. LWT-Food Science and Technology 50:414-419

Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. (2005). Compositional and sensory characteristics of three native sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. J Agric. Food Chem. 53:7586-91.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., & Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chemistry 104, 943–947.

Al-Shahib, W., & Marshall, R. J. (2003). The fruit of the date palm: its possible use as the best food for the future? International Journal of Food Sciences and Nutrition 54(4), 247–259.

Aslam, J., Khan, S. H. & Khan, S. A. (2013). Quantification of water soluble vitamins in six date palm (Phoenix dactylifera L.) cultivar's fruits growing in Dubai, United Arab Emirates, through high performance liquid chromatography. Journal of Saudi Chemical Society (2013) 17, 9-16.

Besbes, S., Drira, L., Blecker, C., Deroanne, C. & Attia H. (2009). Adding value to hard date (*Phoenix dactylifera L.*): compositional, functional and sensory characteristics of date jam, Food Chemistry 112:406–411

El-Nagga, E.A. &Abd El-Tawab. Y.A. (2012). Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. Annuals of Agricultural Sciences 57, 29-36

El Arem Amira, Flamini Guido, Saafi Emna Behija, Issaoui Manel, Zayene Nesrine, Ferchichi Ali, Hammami Mohamed, Helal Ahmed Noureddine, Achour Lotfi. (2001).

Chemical and aroma volatile compositions of date palm (Phoenix dactylifera L.) fruits at three maturation stages. Food Chemistry 127, 1744–1754

Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C. & Drira, N.-E. (2008). Date flesh: chemical composition and characteristics of the dietary fibre. Food Chemistry 111:676–682

El-Sharnouby GA, Aleid SM, Al-Otaibi MM. (2014). Liquid Sugar Extraction from Date Palm (Phoenix dactylifera L.) Fruits. J Food Process Technol. 5:402. doi: 10.4172/2157-7110.1000402

Ismail B, Haffar I, Baalbaki R,Mechref Y and Henry J. (2006). Physico-chemical characteristics and total quality of five date varieties grown in the United Arab Emirates. Int J Food Sci Technol. 41:919–926

Khosravanipour Mostafazadeh, A & Sarshar, M & Javadian, Sh & R. Zarefard, M & Amirifard Haghighi, Z. (2011). Separation of fructose and glucose from date syrup using resin chromatographic method: Experimental data and mathematical modeling. Separation and Purification Technology. 79. 72-78. 10.1016/j.seppur.2011.03.014.

Maffei, M.E. (2010). Changes in biosynthesis of aroma volatile compounds during on-tree maturation of "Pink Lady" apples. *South Afr. J. Bot. 76*:612–631.

Muhammad Umar Nasir1, Sarfraz Hussain, Saqib Jabbar, Farhat Rashid, Nazia Khalid, Arshad Mehmood. (2015). A review on the nutritional content, functional properties, and medicinal potential of dates. SCIENCE LETTERS 3(1),17-22

Ogundiwin, E.A.; Peace, C.P.; Gradziel, T.M.; Parfitt, D.E.; Bliss, F.A.I.; Crisosto, C.H.A. *BMC Genomics.* Fruit quality gene map of *Prunus*. 2009, *10*, 587.

Zhen-Xing Tang, Lu-E Shi and SalahMAleid. J Sci Food Agric.. Date fruit: chemical composition, nutritional and medicinal values, products. 2013 (10):2351-61

Figures

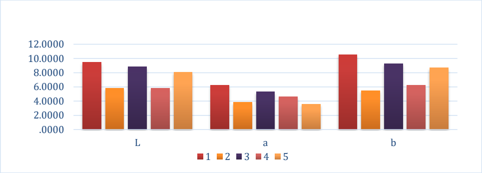

Fig. 1

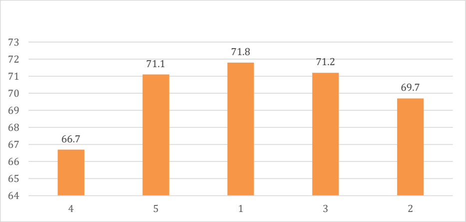

Fig **2** Percentage of total soluble solids in 5 date syrup samples

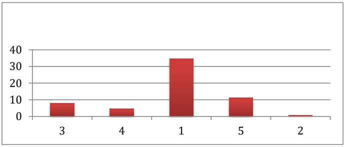

Fig **3** Viscosity of date syrup samples

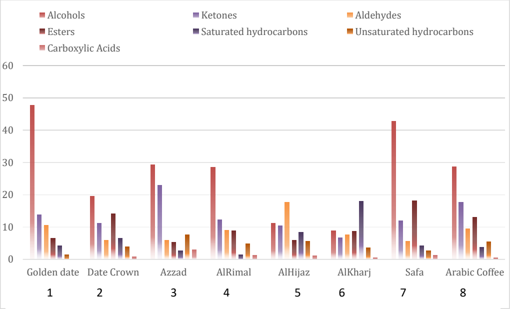

Figure 4 The percentage of volatile chemical groups in date fruit syrup brands

Preventing date palm fraud: towards developing a database for authentication of dates and their geographical origin

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Abstract

Food fraud is a serious problem that is increasing around the world. To prevent date palm fraud, we are developing a spectral database of date palm fruits from around the world, including from United Arab Emirates, Saudi Arabia, Oman etc. Dates from certain areas are highly valued by consumers. For example, dates from Madinah in Saudi Arabia is very popular amongst some people due to the link of this city to the Prophet of Islam. However, consumers may be paying higher price for dates that actually do not originate from Madinah. This not only misleads the consumers but also deprives the farmers their profits. Hence it is necessary to develop a geographical certification of dates so that food fraud is prevented and the rights of the consumers and farmers are protected. We are using spectroscopic and microscopic methods to analyse different types of dates. Spectriscopic methods being used includes Fourier transform infrared spectroscopy along with multivariate statistical analysis to classify and authenticate dates. Inductively coupled mass spectrometry (ICP- MS) is being used to determine the elemental composition of the dates. It is intended to produce a publicly accessible database of dates that could be used by food authorities around the world to authenticate dates and prevent food fraud. There will be potential for researchers to submit their data and download data for further research for improving authentication of dates for the benefit of consumers and date farmers around the world. Latest progress in our research will be presented.

The Date Industry in the United States and Mexico

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Abstract

The date palm industry in the United States and Mexico exists in four states in the Sonora Desert, comprises almost 10,000 ha, and has a farm gate value of more than USD $100,000,000. The industry is small, but exports and imports to a variety of countries around the world. 'Deglet Noor' and 'Medjool' are the major varieties. Some cultural practices are common to other date growing areas, while others are unique to the industry. Strengths of the industry include the varieties grown, the increasing use of value-added products, ample water, pollen, offshoots, the fact that the industry is virtually disease free, the growers are innovative, and growing dates is profitable. Weaknesses include the fact that Americans and Mexicans do not eat many dates, there are only two varieties, the variety of value-added products is limited, labor costs in Mexico are lower than in the US, harvest and thinning mechanization is not well-advanced, and there is a lack of funding for research. Opportunities include subsidies given to Mexican growers to produce dates, improved marketing, the health benefits of dates are becoming increasingly known, and there are several large underdeveloped markets. Threats are from potential insect pests, the South American Palm Weevil, the Pink Hibiscus Mealybug, and the Carob Moth.

Keywords: Location, Production, Trade, Cultural Practices, SWOT analysis

LOCATION AND EXTENT OF THE INDUSTRY

The North American commercial date palm industry is located in the Sonora desert, in the states of California and Arizona within the United States and in the states of Baja California Norte and Sonora within Mexico (Fig. 1). In California, the industry exists two counties comprising four valleys. Within Riverside County, the northernmost valley is the Palo Verde, surrounding Blythe, CA. Continuing southward, there is the Coachella Valley, surrounding Coachella and Indio, CA. Within Imperial County, and south of the Salton Sea lies the Imperial Valley that surrounds El Centro, CA, and finally the Bard Valley which lies north of the Colorado River and is contiguous to Yuma Arizona. In Arizona, the date palm industry exists primarily in the Colorado River valley and tablelands around Yuma, but there are a few orchards scattered east of Yuma, extending as far as 150 km away in the Hyder Valley along the Gila River. In Baja California Norte, the industry exists in the Mexicali Valley that surrounds the city of Mexicali and is an extension of the Imperial Valley, while in Sonora, date palms are grown in the valley and tablelands to the east of the Colorado River. There are also small orchards located in the Mexican states of Baja California Sur and Coahuila, and one orchard located in California's Mohave Desert, but these orchards are isolated and do not produce dates for other than their local markets.

Together, these locations contain almost 9500 hectares of dates in 2016 (Table 1). California accounts for 56% of the total, 34% in Riverside County (Riverside County, 2016) and 22% in Imperial County (Imperial County, 2016). Arizona accounts for 25% (T. Joseph, pers. comm.), and Mexico contributes 19% to the total (SAGARPA 2016a). Production ranges from six to ten MT per hectare, with the lower yields in California and Arizona likely due to the planting of large numbers of young palms that have not yet reached their full bearing potential. It is also possible that hectares of dates in Mexico might be higher than reported and yields might be lower, as the author is uncertain if young palms are included in Mexican statistics. For 2017, the farm-gate value of the total industry totals more than USD $ 100,000,000.

COMPARISON OF THE US DATE INDUSTRY TO WORLDWIDE DATE PRODUCTION AND TRADE

According to the FAO (FAO, 2017), Egypt was the largest date producer in 2016, with about 1.7 million metric tons (MMT) of production (Fig. 2), followed by Iran, Algeria and Saudi Arabia each with about 1.0 MMT. In contrast, the FAO reports that date production in the US was about 38,000 MT, and that of Mexico was about 8,000 MT, totaling about 46,000 MT.

For the US industry, date exports in 2017 were valued at almost USD $8,000,000 (FAS, 2017). Twenty nine percent of the dates were exported to Canada, followed by Australia (27%), the United Kingdom (10%), Mexico (8%), and Indonesia (5%). Twenty one percent were exported to other countries, with no individual country receiving more than 4% of the total.

Date imports into the US in 2017 were valued at about USD $47,000,000. Thirty two percent of the dates were imported from Tunisia, followed by Israel (26%), Mexico (12%), Algeria (10%), and Pakistan (5%). Imports from all other countries totaled 15%.

The US and Mexican industries grow two main varieties, 'Deglet Noor' and 'Medjool'. 'Deglet Noor was first planted in California in the 1902 (Hilgeman, 1972), while 'Medjool' was first planted at an experiment station in California in 1934, following its import in 1927 (Thackery, 1952). The California industry grew beginning in the 1890's, once there were sufficient offshoots of commercially acceptable varieties, (except for 'Medjool' that was initially planted commercially in 1944 (Berryman, 1972)), while the Arizona industry started at about the same time in the Phoenix area, but subsequently declined in the 1950's following crop losses due to rain and freezes, and growing urbanization Wright, 2016). The remaining Arizona industry began to grow about 25 years ago. Mexican commercial production is less than 15years old. Considering the number of new plantings in California, Arizona and Mexico, there will be a 15 to 20% production increase in the next five to 10 years.

Today, there are no US or Mexican statistics as to the exact numbers of trees of each variety, but based on anecdotal evidence, California accounts for almost all the 'Deglet Noor' production, chiefly found in the Coachella Valley, while Medjool predominate everywhere else, especially in the Bard Valley and in Arizona. 'Deglet Noor' probably accounts for about 55 to 60% of US production while 'Medjool' accounts for 40 to 45%. There may be as much as 5% of the production of other varieties in the US, chiefly 'Barhi', and local seedling varieties, such as 'Black Sphinx', "T.R.", and 'Honey'. In Mexico, 'Medjool' is likely the most planted cultivar.

HORTICULTURAL PRACTICES IN THE US AND MEXICAN DATE INDUSTRY

Historically, dates were planted at 9-meter square plantings, although 8-meter by 8-meter plantings are increasingly common today. Occasionally, dates are planted at higher densities if the grower plans to sell trees for landscaping purposes. Considering that both 'Medjool' and 'Deglet Noor' are prolific producers of offshoots, most orchards are planted from offshoots, rather than tissue culture derived palms. Workers remove offshoots from the mother palm and orchards are usually established in May or June, once the soil warms and root growth is assured. Sometimes, offshoots are planted in pots for a year to grow roots before being transplanted to an orchard site.

Irrigation is typically via drip or micro-sprinklers, although flood irrigation is practiced in some areas. Irrigation rates vary depending on tree age, but can be as much as 4000 liters per week in the hottest part of the summer. There are no recommended fertilization rates, but N, K and B are commonly applied with conventional sources. Some organic growers apply solely composted manure, typically up to 20 MT per ha. One male palm is planted for every 49 female palms. Growers collect pollen from the male flowers as soon as the spathes crack, and they often dilute the pollen with flour, corn starch or talc. Sometimes they use last year's pollen that has been stored in the freezer. Pollen is applied multiple times to each female flower using squeeze bulbs or bottles, modified leaf blowers, or larger blowers that can be pulled through the orchard with a tractor. Workers pollinate smaller trees from the ground and use ladders once the trees are too tall to be easily worked from the ground. Once the ladders are too short, growers use vertical mast forklifts and ultimately reach lifts, to which specially designed platforms are attached, to carry groups of 4 to 8 workers into the crowns of the trees. In areas where 'Deglet Noor' predominates, older trees have ladders permanently attached to the trunks several meters above the ground. Workers attached ladders that they carry into the field to the permanent ladders and ascend the trees in that manner.

For Medjools, the fruit is thinned, up to 70% is removed. Each grower has his or her own formula as to how many bunches fruit strands, and fruit to remove. Because it is likely that rain and humidity will cause fermentation in the date bunch at harvest, 'Medjools' must be painstakingly thinned by hand; each date must have some space between it and the adjacent date so there can be sufficient air movement to reduce the chance of fermentation. To further improve air flow, workers insert a galvanized wire spreader ring to spread the strands out. In regions where rain is not a threat during harvest, 'Medjool' thinning can be accomplished by simply cutting the strands, thus reducing strand length without regard to the space between dates. However, that method of thinning for 'Medjool' has not proved effective in the Sonora Desert. 'Deglet Noor' fruit are not thinned, since this variety ripens later, when the chance of rain and humidity is much less. 'Barhi' dates are sometimes thinned for size only, as they are not affected by fermentation when harvested in the khalal stage. Workers pull and tie the 'Medjool' date bunches to leaf stalks below to force the proper curvature of the fruit arm so that they hang below the leaves for ease of harvest. Later, they tie the bunches to leaf stalks above to support the weight of the growing fruit. Finally, they place bags on bunches of 'Medjool' dates about four weeks before harvest. Bags are for protection from birds, to avoid losing fallen fruit, to hasten fruit ripening, to protect the fruit from rain, and to modify the environment around the fruit. In zones with heavier soils, where moisture from the soil may lead to fermentation, growers use white nylon net bags to improve air circulation. In areas with lighter soils, growers use white cotton/nylon bags with tighter mesh to avoid losing moisture. 'Deglet Noor' fruit is bagged with paper or cloth bags to protect the fruit from the rain. Harvest for 'Medjool' dates begins in late August. Since the dates in a bunch do not all ripen at the same time, workers harvest each tree four times, about ten days apart, continuing until early October. 'Deglet Noor' harvest begins in late September. In this case, the entire bunch is cut from the tree and lowered to the ground. 'Barhi' dates are harvested in late July and early August, before they mature into the rutab stage.

SWOT ANALYSIS OF THE US AND MEXICAN DATE INDUSTRY

It is sometimes instructive to analyze an organization or industry according to its strengths, weakness, opportunities and threats; an exercise known as a "SWOT" analysis. Strengths are internal characteristics that are helpful to the organization, while weaknesses are internal characteristics that are harmful. Opportunities are external factors that may be helpful to the organization, while threats are external factors that may be harmful.

Strengths

Certainly, one of the main strengths of the US and Mexican industries is the fact that high quality productive varieties are grown. Medjool is known world-wide as one of the world's premium dates because it is large, soft and sweet. 'Medjool' is used primarily for fresh eating. 'Deglet Noor' is also known world-wide, and is primarily used for fresh eating, baking and value-added products. Value added products are becoming increasingly important in the US. Deglet Noor is commonly used in energy bars; prices paid to growers for 'Deglet Noor' are just as high as that paid to 'Medjhool' growers because of the demand for dates as the key ingredient in those bars. Dates are increasingly found in confections, baked goods and breakfast foods, and are marketed as natural and healthy.

Another strength of the industry is an ample water source, chiefly the Colorado River. Despite several years of drought that threaten to limit water deliveries to some parts of the US, the date industry is not in those areas that would be affected by reduced water deliveries. Cost of water in Arizona and Mexico is relatively low (in California water cost is higher), because it is subsidized by the government. In many cases, surface water flood irrigation delivery systems are already built. Where well water is used, the cost of the well drilling and installation of a pressurized system can be high, but then the only typical reoccurring cost is for the electricity needed to pump the water. Some growers are converting from flood to pressurized systems to more efficiently deliver water and fertilizer to the trees on an as-needed basis.

Another strength is ample pollen availability. Because both the 'Medjool' and 'Deglet Noor industry is so well-established, growers have been able to propagate many male plants so that everyone has adequate pollen sources. Also, since most of the industry is one of two varieties, growers are relatively certain of the origin of their pollen.

The US and Mexico are free of Fusarium oxysporum f. sp. albedinis (Bayoud disease), due to the USDA quarantine in effect since 1929. As a result, there are no significant fungal problems affecting dates grown in the US or Mexico. Furthermore, there are no significant insect pests of dates grown in Arizona or Mexico. California is threatened by insect pests as noted below.

Use of high technology is increasing in the US date industry. As mentioned previously, pollination with blowers is common, and growers are experimenting with the use of drones to pollinate dates. Growers are also developing their own harvest aids to hake harvesting more efficient and cost-effective.

Growing dates in the US is still profitable. Growers paid from USD \$1.65 to \$3.30 per kg of fruit, and the yield for a hectare of date palms is about 11,200 kg. For a mature orchard, the gross returns would be USD \$18,500 to \$37,000 per hectare, while growing and harvesting costs are about \$14,000 per hectare.

Weaknesses

Probably the main weakness of the industry is the fact that Americans and Mexicans eat relatively few dates, compared with other regions of the world. It is estimated that the typical American consumer eats less than 500 g per person per year (D. Mansheim, pers. Comm), because there are many other choices of fresh fruit. There is no real tradition of eating dates, unlike that of Middle Eastern countries. In fact, some people confuse dates with figs! Only two varieties are typically available, excepting Medjool and Deglet Noor. Marketing has increased consumption, but it is not too innovative, and some value-added date products are virtually unknown in the US, such as date syrup, date sugar, and date spread. Dates filled with nuts or candied citrus fruit peel are virtually unknown, and dates stuffed with peanut butter or cheese, and wrapped in bacon are available from only one supplier.

Another weakness is the higher growing costs in the US versus Mexico that are mostly due to lower labor costs in Mexico (Figure 3). This weakness may be overcome by increased use of mechanization, yet there is not yet any mechanical harvesting or thinning in the US or Mexico. Furthermore, there is little consistent funding for research. Grower funding is not often available, and researchers must compete with other crops for funding.

Opportunities

The Mexican government subsidizes planting of dates (SAGARPA, 2016b), which is an opportunity for those growers and for palm tree suppliers. There is a high-quality tissue culture lab in Santa Ana, CA., which has been supplying much of the Mexican industry. With this lab in place, supply of lesser-known dates will increase as propagative capacity grows. Even though the lack of mechanical harvesting dates is a weakness, many other crops in the US, such as nuts, that are harvested mechanically. This would suggest that mechanical harvesting is not likely to be particularly difficult to achieve.

Marketing is increasing awareness of the date in the US, with a goal of increasing consumption. Traditionally, dates were marketed in 2.5 and 5 kg boxes, but now dates are increasingly marketed in smaller, consumer-friendly plastic containers. These containers often have recipes attached, or provide a link to a website, blog or Facebook page that have additional information on how to use and consume dates. Dates have traditionally been marketed as conventionally grown, but now are available as organic fruit. Natural Delights, Sun Maid and others market in the US, with availability almost year-round.

Within the US and Canada, dates are marketed as a superfood, containing 16 vitamins and minerals, and 50% more potassium by weight than bananas. Consumers are learning that dates are a good source of fiber and antioxidants and are cholesterol and fat free.

Outside the US, date producers have identified several underdeveloped markets. These include China, India and S.E. Asia. If US and Mexican producers can market their high-quality products in these countries, it would be very advantageous.

Threats

The most pressing threat to the US and Mexican date industry is the South American palm weevil (SPW) (*Rhynchophorus palmarum*), native to South and Central America, which has been found in Northwest Mexico and Southwest United States (CISR, 2018). The SPW is related to the red palm weevil (RPW) but is slightly larger and is typically black. SPW lifecycle is like that of the RPW, and it can fly several kilometers. Like the RPW, SPW larvae feed inside the palm crown and the damage kills the tree. Additionally, the SPW can carry the red ring nematode, *Bursaphelenchus cocophilus*, which has not yet been found in the United States but is found in southern Mexico. This nematode can lead to serious economic losses of commercial coconut and oil palm plantations in Central and South America and can also damage or kill landscape palms. SPW currently infests Canary Island date palms *(Phoenix canariensis*), and poses a threat to other palms, including the common date palm *(P. dactylifera*) orchards in the region. Estimated SPW populations in *P. canariensis* in San Diego have increased greatly in the past year and over 300 trees have been infested and many killed. Reports indicate that hundreds of palms have been killed in Mexico. Only one weevil has been found in Arizona. Drones are being tested and evaluated to identify infested trees. Trapping and properly applied pesticides are some of the control measures that can be taken. Semiochemicals are being developed that will attract the SPW to small, but lethal doses of a pesticide that is applied to the tree as a small mass of paste. SPW repellants are also being developed.

Another threat is the Pink Hibiscus Mealy Bug, *Maconellicoccus hirsutus,* which was found in California in 1999(CDFA, 2018). This insect feeds on the soft tissues of the date palm and its toxic saliva causes deformation of the bud and emerging leaves. Honeydew excreted by the insect also encourages the formation of sooty mold. Currently, biocontrol methods are being developed to control this insect.

A final threat is the Carob Moth, Ectomyelois ceratoniae. Carob moth infests primarily Deglet Noor dates. Sex pheromones are being deployed as mating disruption to control this pest (Vetter et al., 2006).

LITERATURE CITED

Berryman, E. 1972. The Medjool date production in Bard, California, Report of the Date Grower's Institute 49:10.

CDFA, 2018. Biological Control of Pink Hibiscus Mealybug. https://www.cdfa.ca.gov/plant/ipc/ biocontrol/83pinkhibiscusmealybug.htm

CISR – Center for Invasive Species Research, 2018. https://cisr.ucr.edu/

FAO, 2017. http://www.fao.org/faostat/en/

FAS, 2017. https://apps.fas.usda.gov/gats/default.aspx

Hilgeman, R.H. 1972. History of date culture and research in Arizona. Report of the Date Grower's Institute 49:11–14.

Imperial County Agriculture Commissioners Report, 2016. www.co.imperial.ca.us/ag/.

Riverside County Agriculture Commissioners Report, 2016. http://www.rivcoawm.org/.

SAGARPA, 2016a. http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ AvanceNacionalSinPrograma.do

SAGARPA, 2016b. http://www.sagarpa.gob.mx/Delegaciones/bajacalifornia/ Boletines/Paginas/045B2016.aspx.

Thackery, F.A. 1952. A few notes on the 'Medjool' date during its isolation in Nevada. Report of the Date Grower's Institute 29:8–10

Vetter, R.S. & Millar, J.G. & Vickers, N.J. & Baker, Thomas. (2006). Mating disruption of carob moth, *Ectomyelois ceratoniae*, with a sex pheromone analog. Southwestern Entomologist. 31. 33-47.

Wright, G.C. 2016. The Commercial Date Industry in the United States and Mexico. HortScience 51:1333-1338.CISR

Table

Table 1. Area of Production, Yield and Farm Gate Value of the Date Palm Industry in the United States and Mexico

Location	**Production Area (Hectares)**	**Yield (MT/ha)**	**Farm Gate Value (USD x 1000)**
California Riverside	3222	6.16	40,110
California Imperial	2136	6.16	19,069
Arizona	2378	7.06	21,098
Mexico – Baja California Norte	841	7.54	10,808
Mexico - Sonora	922	9.81	10,358

Figures

Figure 1. *Major date growing areas in the United States and Mexico. Areas within California are shown in green, within Arizona in blue, within Sonora in orange and within Baja California in red.*

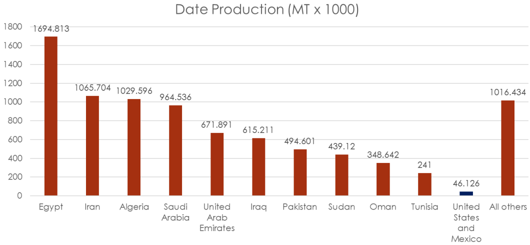

Figure 2. Date production of selected countries in 2016

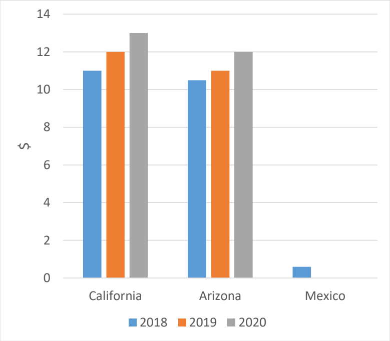

Figure 3. Minimum wage in the US and Mexico – 2018-2020.

Preliminary evaluation of palm date (*Phoenix dactylifera L.*) fruit juice in production of biosurfactant by *Pseudomonas aeruginosa* isolated from fuel-contaminated soil

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Abstract

Surfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases with different degrees of polarity. These molecules reduce the surface tension and interfacial tension and create microemulsions. Biosurfactants are produced by bacteria or yeast from various substrates including sugars, glycerol, oils, hydrocarbons and agricultural wastes. Biosurfactants are of interest because of their diversity, possibility of large-scale production and environmental protection. The choice of inexpensive raw materials is important to overall economy of the process because often, the amount and type of a raw material can contribute considerably to the production cost. There are few reports on biosurfactants production using inexpensive raw materials as substrates. Therefore, the objective of this work was to study the biosurfactant production by *Pseudomonas aeruginosa* using crude juice of dates as carbon source. On the other hand, the biosurfactant produced by the isolate was tested as bio-stimulant for the growth of lentil plant (*Lens culinaris*), and as antimicrobial against two standard pathogen strains (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300) and against three pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*). Besides, the crude biosurfactant extract obtained was analyzed using Fourier Transform infrared (FTIR) technique. The crude juice was prepared from ripened dates of low quality called H'Chef. The bacterial strain capable of producing biosurfactants was isolated from gas station soil contaminated with fuel (located in Boumerdès, Algeria) by selective enrichment culture technique. The results showed that the growth of the isolate on medium containing glucose as a sole carbon source gave a foaming value of 4.8 % and cleaning activity of 40 % after 112h of incubation, while the growth of the isolate medium containing date juice, permitted an improvement of both foaming test (36 %), and cleaning activity (100 %). On the other hand, biosurfactant produced by the isolate on date juice showed antibacterial activity against pathogenic bacteria: *Escherichia coli*, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus*, and was capable of stimulating lentil growth. Besides, date juice medium yielded around 6 g/L of biosurfactant in 112 h. However, extensive research is needed to establish the suitability of this low-cost substrate in industrial-level biosurfactant production process. The FTIR analysis showed that the strain produced rhmnolipids in medium.

Evaluation of date palm(***Phoenix* *dactylifera*** L.) production and soil properties in relation to the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara area of The State of Kuwait

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Abstract

An experiment was conducted at Amghara Station of The State of Kuwait on barhee variety of date palm with ten treatment combinations involving different sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils during October, 2013 to September, 2016. The experimental design adopted was randomized complete block with five replications. The results showed that the Date palm tamar fruit yield increased with increasing rates of sewage sludge and compost manure as compared to control. The influence of sewage sludge in conjunction with arbascular mycorrhizal fungi(AMF) on the tamar yield of crop was more pronounced than that of compost manure. Such an increase in the yield of tamar may be attributed to the improvement in the fruit yield parameters such as number of fruiting branches and fruits per bunch; weight, length and girth of fruit. This may further be related to the maintenance of higher moisture and availability of nutrients in the soil. On an average over 3 years, 10 t/ha of sewage sludge(71.97 t/ha or 22.57% increase) or compost manure(70.00 t/ha or 19.31% increase) along with AM fungi over control(58.43 t/ha) could be recommended to date palm crop in order to increase the yield levels and fertility status especially the organic matter content of the soil.

Keywords: Organic manure, sewage sludge, compost manure, date palm, tamar yield.

INTRODUCTION

Date palm is one of the most important dry fruit crops grown under the hot arid conditions in Kuwait. Since the soils are sandy in nature, the growth of date palm is highly affected by organic matter content, water and nutrient availability in the soil.

Sewage sludge can substitute for commercial fertilizers and organic matter, if applied in the right amounts to soil. By using sludge, it is a possibility for farmers to supply their lands with organic fertilizer at low costs. Therefore, recycling of sludge for agricultural purpose seems to be an appealing solution for sustainable management of sludge. Sewage sludge (bio-solids), which is enriched in nitrogen, phosphorus, organic matter and other trace elements, represents a good source of nutrients for plant growth and a good soil conditioner to improve soil physical properties. Due to its high organic matter content, sewage sludge can improve physical, chemical, and biological properties of soil (Alcantara et al. 2009). Thus, sludge application helps to reduce soil erosion and improves the soil quality as a plant growth medium. The fertilizer effect enables a reduction in cost for nitrogen and phosphorus mineral fertilizers and may improve crops yield on sludge treated fields (Wild and Jones, 1991; Petersen et al. 2003).

According to the study by Veysel Saruhan et al. (2010), sewage sludge when applied to the soil with increasing amounts raised the mineral matter content of plant and resulted in the increase of growth and herbage yield of bird's foot trefoil. Heavy metal contents were below the critical levels in the soil and plant. Ma. del Mar Delgado Arroyo et al. (2002) reported that the application of sewage sludge compost @ 12 t/ha plus 350 kg urea/ha resulted in 20% greatest growth of maize than the mineral fertilization. No toxic effects arising from the heavy metals in the plant were observed. Moreover, the concentration of heavy metals in the soil was below Spanish and European legal limits.

Considerable research has been accomplished worldwide on the use of sewage sludge on soil and crop. In many investigations with different climatic and soil conditions have reported a substantial increase in plant growth, crop yield and biomass production upon sewage sludge application (Samaras et al. 2008 and Togay et al. 2008). Using organic wastes such as sewage sludge, compost and cow manure is a proper practice for returning organic matter and some nutrients into the soil, particularly in arid and semi-arid regions. Application of sewage sludge, compost and cow manure at 25 Mg/ha significantly increased available Fe and Zn of the soil. The application of organic wastes increased the dry matter yield of the corn, alfalfa and targetes flower plants(Sharifi et al. 2011).

Application of FYM with higher fertilizer level has given maximum tuber yield of potato in sandy clay loam soil (Krishnamurthy *et al*., 1999). Poultry waste compost under VAT method is Agronomically efficient in sunflower production (Jayabharat Reddy *et al*., 2000). Application of organic manure 25 t/ha resulted in significantly higher tuber yield of potato (27.9%) as compared to 12.5 t/ha(Abd Al Khalak Abd Al Gaffar *et al*., 2007).

This study was conducted in order to evaluate date palm(*Phoenix* *dactylifera* L.) production and soil properties in relation to the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara area of The State of Kuwait.

MATERIAL AND METHODS

An experiment was conducted at Amghara Station of The State of Kuwait on barhee variety of date palm with ten treatment combinations involving different sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils during October, 2013 to September, 2016. The experimental design adopted was randomized complete block with five replications. The treatment combinations included in the experiment were as detailed below.

F1 – Control
F2 – Arbascular Mycorrhizal Fungi(AMF)
F3 – Sewage sludge @ 5 t/ha
F4 – Sewage sludge @ 5 t/ha + AMF
F5 – Sewage sludge @ 10 t/ha
F6 – Sewage sludge @ 10 t/ha + AMF
F7 – Compost manure @ 5 t/ha
F8 – Compost manure @ 5 t/ha + AMF
F9 – Compost manure @ 10 t/ha
F10 – Compost manure @ 10 t/ha + AMF

The sandy loam soil at Amghara initially had a pH of 7.52, EC 9.48 dSm^{-1} , OM 0.29%, PO_4 4.24 ppm, K 1.91 meq/l, Ca 24.76 meq/l, Mg 13.22, Na 77.73 meq/l, Cl 78.07 meq/l, HCO_3 3.66 meq/l, SO_4 36.19 meq/l, and B 1.08 ppm(Table 1).

Soil sampling was done from 2 depths (0-25cm & 25-50cm) for initial physical, chemical & microbial analysis. Sewage sludge & organic manure with and without mycorrhiza were incorporated as per the treatments @ 5 or 10 t/ha/year in October by opening a furrow to a depth of 15-20cm around each tree in the basin. The organic manure with or without mycorrhiza were mixed well and covered. Mycorrhizal inoculum(*Glomus intraradices* L.) procured from Germany was used in the experiment.

Compost manure had a pH of 7.2, O.M. 45%, N 2.3%, P2O5 1.01% & K 0.63 meq/l(Table 2). Sewage sludge manure had a pH of 8.88, O.M. 14.3%, N 1.002%, P2O5 1.63% & K 16 meq/l with a moisture content of 34.86%(Table 3).

First and second split dose of N, P_2O_5 and K_2O complex fertilizer(18-18-18 + TE) @ 1 kg//tree was applied in to the small furrows opened 5-10 cm deep around each date palm tree in the basin during the months of December and February. Micro nutrient mixture in the form of micromel combi @ 100g/tree was also applied during February. Later the fertilizers were mixed well in the soil and covered. Whereas, the third split dose of complex fertilizer(20-20-20 + TE) @ 1 kg//tree along with Potassium Sulphate @ 200 g/tree was applied during the month of April. After each application. irrigation was given to dissolve the fertilizer which helps in further uptake of nutrients by date palm crop.

Fourth stage treated sewage water was used for irrigation and it had a pH of 7.5, EC 0.25 dSm^{-1}, TDS 160 ppm, K 0.3 meq/l, Na 1.1 meq/l, Mg 0.2 meq/l , Ca 1.6 meq/l, HCO_3 1.2 meq/l, Cl 1.6 meq/l and SO_4 0.4 meq/l. Total Coliforms count was 140 MPN/100 ml(Table 4). Bubbler irrigation was scheduled to the crop as per the requirement of the trees depending upon the climatic conditions and growth stages as detailed below:

Month	Water Requirement (Imperial Gallons/day)	Irrigation Interval (days)
January	10	8
February	12	8
March	18	4
April	24	4
May	32	3
June	40	2
July	40	2
August	36	2
September	28	3
October	20	4
November	14	8
December	10	8

All the crop management practices were adopted as per the recommendation. The necessary data on soil properties, soil moisture, irrigation water and biometrics were recorded. The tamar fruit yield data was subjected to statistical analyses as per standard procedure and presented.

RESULTS AND DISCUSSION

The results obtained and the discussion on the evaluation of date palm(*Phoenix dactylifera* L.) production and soil properties in relation to the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara area of The State of Kuwait during 2013-2014, 2014-2015, 2015-2016 and the average of 3 years is presented in tables 5 to 8, 8a & 8b and graphically represented also in figures.

Use of sewage sludge manure is economically advantageous to compost manure as the sewage sludge manure is locally available from the waste water treatment plants in Kuwait & hence the cost involved will be reduced. Hence, either compost or sewage sludge manure could be used favourably in the sandy soils of Kuwait.

The results showed that the Date palm tamar fruit yield increased with increasing rates of sewage sludge and compost manure as compared to control. The influence of sewage sludge in conjunction with arbascular mycorrhizal fungi(AMF) on the tamar yield of crop was more pronounced than that of compost manure. Such an increase in the yield of tamar may be attributed to the improvement in the fruit yield parameters such as number of fruiting branches and fruits per bunch; weight, length and girth of fruit(Table 8a & 8b). This may further be related to the maintenance of higher moisture and availability of nutrients in the soil(Table 9). Similar results were also reported by other workers in different crops(Abdul Khalak and Kumaraswamy, 1993; Veeranna *et al*., 2001 and Abd Al Khalak Abd Al Gaffar *et. al.* 2007).

On an average over 3 years, 10 t/ha of sewage sludge(71.97 t/ha or 22.57% increase) or compost manure(70.00 t/ha or 19.31% increase) along with AM fungi over control(58.43 t/ha) could be recommended to date palm crop in order to increase the yield levels and fertility status especially the organic matter content of the soil.

Similarly, according to the study by Veysel Saruhan et al. (2010), sewage sludge when applied to the soil with increasing amounts raised the mineral matter content of plant and resulted in the increase of growth and herbage yield of bird's foot trefoil. Heavy metal contents were below the critical levels in the soil and plant. Ma. del Mar Delgado Arroyo et al. (2002) reported that the application of sewage sludge compost @ 12 t/ha plus 350 kg urea/ha resulted in 20% greatest growth of maize than the mineral fertilization. No toxic effects arising from the heavy metals in the plant were observed. Moreover, the concentration of heavy metals in the soil was below Spanish and European legal limits.

The motivation for recycling of sewage sludge to agricultural soil is the low cost of this disposal method, the soil organic matter preservation effect and the fertilization effect. Sewage sludge (biosolids), which is enriched in nitrogen, phosphorus, organic matter and other trace elements, represents a good source of nutrients for plant growth and a good soil conditioner to improve soil physical properties. Due to its high organic matter content, sewage sludge can improve physical, chemical, and biological properties of soil (Alcantara et al. 2009).

Thus, sludge application helps to reduce soil erosion and improves the soil quality as a plant growth medium. The fertilizer effect enables a reduction in cost for nitrogen and phosphorus mineral fertilizers and may improve crops yield on sludge treated fields (Wild and Jones, 1991; Petersen et al. 2003). Application of sewage sludge, compost and cow manure at 25 Mg/ha significantly increased available Fe and Zn of the soil(Sharifi et al. 2011). Sewage sludge can substitute for commercial fertilizers and organic matter, if applied in the right amounts to soil. By using sludge, it is a possibility for farmers to supply their lands with organic fertilizer at low costs. Therefore, recycling of sludge for agricultural purpose seems to be an appealing solution for sustainable management of sludge.

In the present study, the date palm crop has responded well to the application of both the sources of organic manure at with the increasing rates of their application in sandy soils of Kuwait. However, the influence of especially the sewage sludge manure addition at 10 t/ha was more pronounced than compost manure. Likewise, considerable research has been accomplished worldwide on the use of sewage sludge on soil and crop. In many investigations with different climatic and soil conditions have reported a substantial increase in plant growth, crop yield and biomass production upon sewage sludge application (Samaras et al. 2008 and

Togay et al. 2008). Using organic wastes such as sewage sludge, compost and cow manure is a proper practice for returning organic matter and some nutrients into the soil, particularly in arid and semi-arid regions. The application of organic wastes increased the dry matter yield of the corn, alfalfa and targetes flower plants(Sharifi et al. 2011).

According to the study by Veysel Saruhan et al. (2010), sewage sludge when applied to the soil with increasing amounts raised the mineral matter content of plant and resulted in the increase of growth and herbage yield of bird's foot trefoil. Heavy metal contents were below the critical levels in the soil and plant. Ma. del Mar Delgado Arroyo et al. (2002) reported that the application of sewage sludge compost @ 12 t/ha plus 350 kg urea/ha resulted in 20% greatest growth of maize than the mineral fertilization. No toxic effects arising from the heavy metals in the plant were observed. Moreover, the concentration of heavy metals in the soil was below Spanish and European legal limits.

Several earlier workers (Krishnamurthy *et al*., 1999 and Jayabharat Reddy *et al*., 2000 and Abd Al Khalak Abd Al Gaffar *et. al*., 2007) also reported that the application of higher quantities of organic manure is responsible for the improvement in the growth and yield of potato and sunflower crops. Since the soils are sandy and poor in organic matter content, incorporation of sunflower plants after harvest organic manure @ 25t/ha and enriches soil fertility and addition of organic manures sustains sunflower production for long time in Kuwait. Veeranna *et al*. (2001) have also revealed similar results and quoted that potato production can be sustained over a long time by maintaining the soil moisture and nutrient status.

SUMMARY AND CONCLUSION

- It is possible to grow successful crop of Date palm on sandy loam soils of Kuwait in the open field under bubbler irrigation by adopting proper management practices.
- The influence of addition of sewage sludge manure to the Date palm crop was more pronounced than compost manure, as it was largely responsible for the improvement in the physical, chemical and biological properties of the soil.
- Since the soils are sandy, poor in organic matter and nutrient content, it is advisable to apply organic manure in the form of sewage sludge(71.97 t/ha or 22.57% increase in yield) or compost manure(70.00 t/ha or 19.31% increase in yield) when applied @ 10 t/ha along with Mycorrhiza and the recommended dose of fertilizers gives higher Date palm tamar fruit yield.
- Addition of organic manure in the form of sewage sludge/ compost manure enriches soil fertility which in turn also helps in sustaining the production for long time in Kuwait.

References

Abd Al Khalak Abd Al Gaffar, Muhammad Abd Allah, Khaled Al Tuwaijery, Swad Al Kandry, Marwa Al Taher and Sanjeev S. Kurup, 2007, Effect of conjunctive use of irrigation water and organic manure on sustainable production of potato var. spunta under drip fertigation in Kuwait. Proj. Rep.(2004-2006), Agriculture Affairs & Fish Resources Authority, Kuwait. p. 30.

Alcantara, S., Pérez, D.V., Almeida, R.A., Silva, G.M., Polidoro, J.C., Bettiol, W., 2009. Chemical changes and heavy metal partitioning in an oxisol cultivated with maize (Zea mays L.) after 5 years disposal of a domestic and an Industrial sewage sludge. Water, Air, and Soil Pollution 203, 3-16.

Jayabharat Reddy, P., Sharanappa, Bandi, A.G. and Abdul Khalak, 2000, Efficacy of poultry waste composts in sunflower(Helianthus annus L.). In. Proc. Natl. Symp. on Agronomy:

Challenges and Strategies for the New Millennium held at Junagadh, Nov. 15-18, 2000, p. 97.

Krishnamurthy, N., Abdul Khalak, Hunsigi, G., Basavaraj, H.K., Prasanna, K.P.R. and Shivaraj, B., 1999, Ware potato production through integrated nutrient supply and management (INSM) in alfisols. In. Proc. of Global Conf. on Potato p. 118, New Delhi, Dec. 6-11. 1999.

Ma. del Mar Delgado Arroyo, Miguel Angel Porcel Cots, Rosario Miralles de Imperial Hornedo, Eulalia Ma. Beltran Rodriguez, Luisa Beringola and Jose Valero Martin Sanchez, 2002, Sewage slude compost fertilizer effect on maize yield and soil heavy metal concentration. Rev. Intl. Contam. Ambient 18(3):147-150.

Mehmet Arif Özyazıcı, 2013, Effects of sewage sludge on the yield of plants in the rotation system of wheat-white head cabbage-tomato. Eurasian J. Soil Sci. 2:35 – 44.

Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sorensen, J., Madsen, T., Petersen, J., Grøn, C., 2003. Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. Soil & Tillage Research 72, 139-152.

Samaras, V., Tsadilas, C.D., Stamatiadis, S., 2008. Effects of repeated application of municipal sewage sludge on soil fertility, Cotton Yield, and Nitrate Leaching. Agronomy Journal 100 (3), 477-483.

Sharifi, M., Afyuni, M., Khoshgoftarmanesh., A. H., 2011, Effects of Sewage Sludge, Compost and Cow Manure on Availability of Soil Fe and Zn and their Uptake by Corn, Alfalfa and Tagetes Flower. JWSS - Isfahan University of Technology. 15 (56) :141-154.

Togay, N., Togay, Y., Doğan, Y., 2008. Effects of municipal sewage sludge doses on the yield, some yield components and heavy metal concentration of dry bean (Phaseolus vulgaris L.). African Journal of Biotechnology 7(17), 3026-3030.

Wild, S.R., Jones, K.C., 1991. Organic contaminants in wastewaters and sewage sludges: Transfer to the environment following disposal. In: Jones KC (ed), Organic Contaminants in the Environment – Environmental pathways & Effects. Elsevier, London, pp 133-158.

Veeranna, H.K., Abdul Khalak and Sujith, G.M. 2001, Effect of irrigation and fertigation methods on sustainable chilli production. In. Proc. Interntl. Res. Symp. On Sustainable Agric. Devt., March 27, 2001 at Bangalore, Part 3: 14.

Veysel Saruhan, Ismail Gull and Isyl Aydin, 2010, The effects of sewage sludge used as a fertilizer on agronomical and chemical features of bird's foot trefoil(Lotus corniculatus L.) and soil pollution. Sci. Res. Essays 5(17):2567-2573.

Tables

Table 1 Initial soil nutrient status of ICARDA Date palm organic manures and micorrhiza experimental site at Amghara Station, Kuwait during October, 2013.

Parameter	Unit	Soil depth(cm)		
		0-25	**25-50**	**Mean**
1. pH	-	7.52	7.52	7.52
2. ECe	dSm^{-1}	12.28	6.68	9.48
3. K	meq/l	3.04	0.78	1.91
4. O.M.	%	0.37	0.22	0.29
5. Ca	meq/l	28.32	21.80	24.76
6. Mg	meq/l	16.44	10.00	13.22
7. Na	meq/l	106.32	49.14	77.73
8. Cl	meq/l	105.32	50.82	78.07
9. HCO_3	meq/l	4.00	3.32	3.66
10. SO_4	meq/l	44.80	27.58	36.19
11. PO_4	ppm	7.20	1.28	4.24
12. B	ppm	1.00	1.15	1.08

Table 2: Chemical analysis of the compost manure used in the ICARDA experimental site at Amghara Station, Kuwait.

Parameter	Unit	Value
1. pH	-	7.20
2. O.M.	%	45.0
3. N	%	2.30
4. P2O5	%	1.01
5. K	meq/l	0.63
6. Cu	ppm	0.15
7. Mn	ppm	0.50
8. Mg	ppm	2.70
9. Fe	ppm	1.10
10. Zn	ppm	0.71

Table 3: Chemical analysis of the sewage sludge used in the ICARDA experimental site at Amghara Station, Kuwait.

Parameter	Unit	Value
1. pH	-	8.88
2. EC 1:5	dSm^{-1}	1.79
3. MC	%	34.86
4. OM	%	14.30
5. N	%	1.002
6. P2O5	%	1.63
7. Na	meq/l	2.93
8. K	meq/l	16.00
9. Cu	ppm	0

10. Mn	ppm	0.554
11. Cd	ppm	0.10
12. Zn	ppm	0.78
13. Fe	ppm	7.50
14. Ni	ppm	0.57
15. Pb	ppm	3.00
16. E. coli	MPN/ g	240
17. TotalColiforms	MPN/ g	135

Table 4: Chemical and microbial analyses of irrigation water(fourth stage treated sewage water) used in the ICARDA experimental site at Amghara Station, Kuwait.

Parameter	**Unit**	**Value**
1. pH	-	7.5
2. EC	dSm^{-1}	0.25
3. TDS	ppm	160
4. Na	meq/l	1.1
5. K	meq/l	0.3
6. Ca	meq/l	1.6
7. Mg	meq/l	0.2
8. CO3	meq/l	-
9. HCO3	meq/l	1.2
10. Cl	meq/l	1.6
11. SO4	meq/l	0.4
12. Total Coliforms	MPN/100 ml	140

Table 5: Fruit (tamar) yield of date palm as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait during **2013-2014.**

Treatment	**Tamar yield (kg/tree)**	**Tamar yield (kg/donum)**	**Tamar yield (tons/ha)**	**Increase over control(%)**
F1 – Control	37.6	767.3	7.67	-
F2 – Arbascular Mycorrhizal Fungi(AMF)	38.4	783.7	7.84	2.22
F3 – Sewage sludge @ 5 t/ha	40.0	816.3	8.16	6.39
F4 – Sewage sludge @ 5 t/ha + AMF	42.4	865.3	8.65	12.78
F5 – Sewage sludge @ 10 t/ha	40.8	832.7	8.33	8.61
F6– Sewage sludge @ 10 t/ha + AMF	44.8	914.3	9.14	19.17
F7 – Compost manure @ 5 t/ha	40.0	816.3	8.16	6.39
F8 – Compost manure @ 5 t/ha + AMF	41.6	849.0	8.49	10.69
F9 – Compost manure @ 10 t/ha	40.8	832.7	8.33	8.61
F10 – Compost manure @ 10 t/ha + AMF	44.0	898.0	8.98	17.08
S.Em.+_	3.867	78.918	0.789	-
C.D. at 5%	NS	NS	NS	-
Mean	**41.04**	**837.5**	**8.38**	**-**

Table 6: Fruit (tamar) yield of date palm as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait during **2014-2015.**

Treatment	Tamar yield (kg/tree)	Tamar yield (kg/donum)	Tamar yield (tons/ha)	Increase over control(%)
F1 – Control	58.6	1196	11.96	-
F2 – Arbascular Mycorrhizal Fungi(AMF)	61.3	1251	12.51	4.60
F3 – Sewage sludge @ 5 t/ha	65.5	1337	13.37	11.79
F4 – Sewage sludge @ 5 t/ha + AMF	67.1	1370	13.70	14.55
F5 – Sewage sludge @ 10 t/ha	67.7	1382	13.82	15.55
F6– Sewage sludge @ 10 t/ha + AMF	72.8	1486	14.86	24.25
F7 – Compost manure @ 5 t/ha	64.5	1317	13.17	10.12
F8 – Compost manure @ 5 t/ha + AMF	66.6	1359	13.59	13.63
F9 – Compost manure @ 10 t/ha	66.3	1353	13.53	13.13
F10 – Compost manure @ 10 t/ha + AMF	70.0	1429	14.29	19.48
S.Em.+_	2.684	58.449	0.585	-
C.D. at 5%	8.052	175.347	1.753	-
Mean	**66.04**	**1348**	**13.48**	**-**

Table 7: Fruit (tamar) yield of date palm as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait during **2015-2016.**

Treatment	Tamar yield (kg/tree)	Tamar yield (kg/donum)	Tamar yield (tons/ha)	Increase over control(%)
F1 – Control	79.1	1614	16.14	-
F2 – Arbascular Mycorrhizal Fungi(AMF)	83.9	1712	17.12	6.07
F3 – Sewage sludge @ 5 t/ha	88.6	1808	18.08	12.02
F4 – Sewage sludge @ 5 t/ha + AMF	91.5	1867	18.67	15.68
F5 – Sewage sludge @ 10 t/ha	90.4	1845	18.45	14.31
F6– Sewage sludge @ 10 t/ha + AMF	98.3	2006	20.06	24.29
F7 – Compost manure @ 5 t/ha	86.7	1769	17.69	9.60
F8 – Compost manure @ 5 t/ha + AMF	88.4	1804	18.04	11.77
F9 – Compost manure @ 10 t/ha	86.9	1774	17.74	9.91
F10 – Compost manure @ 10 t/ha + AMF	96.0	1959	19.59	21.38
S.Em.+_	3.176	64.816	0.648	-
C.D. at 5%	9.528	194.447	1.945	-
Mean	**88.98**	**1816**	**18.16**	**-**

Table 8a: Moisture content and yield parameters of date palm fruits as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait(average of 3 years).

Treatment	Moisture content of fruits at Khalal stage(%)	Fruit bunch wt(kg)	Fruit stalk wt(kg)	Fruit wt(kg)	No. of fruits/ bunch
F1 – Control	23.0	7.60	0.50	7.10	738
F2 – Arbascular Mycorrhizal Fungi(AMF)	24.4	8.15	0.75	7.40	780
F3 – Sewage sludge @ 5 t/ha	35.0	9.60	0.65	8.95	1087
F4 – Sewage sludge @ 5 t/ha + AMF	43.9	10.50	0.80	9.70	1189
F5 – Sewage sludge @ 10 t/ha	40.1	10.25	0.63	9.62	1130
F6– Sewage sludge @ 10 t/ha + AMF	47.3	11.20	0.70	10.50	1423
F7 – Compost manure @ 5 t/ha	25.6	9.10	0.45	8.65	930
F8 – Compost manure @ 5 t/ha + AMF	36.9	9.95	0.56	9.39	1094
F9 – Compost manure @ 10 t/ha	29.6	9.25	0.55	8.70	1045
F10 – Compost manure @ 10 t/ha + AMF	46.2	11.00	0.65	10.35	1320
Mean	**35.2**	**9.50**	**0.63**	**8.87**	**1074**

Table 8b: Fruit yield parameters of date palm fruits as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait (average of 3 years).

Treatment	No. of fruit branches/ bunch	Weight/ fruit (g)	Fruit length (cm)	Fruit girth (cm)
F1 – Control	64	7.72	2.42	1.90
F2 – Arbascular Mycorrhizal Fungi(AMF)	67	8.68	2.57	1.92
F3 – Sewage sludge @ 5 t/ha	68	9.15	2.68	1.97
F4 – Sewage sludge @ 5 t/ha + AMF	80	9.95	2.77	2.07
F5 – Sewage sludge @ 10 t/ha	71	9.85	2.77	2.03
F6– Sewage sludge @ 10 t/ha + AMF	88	11.10	2.93	2.18
F7 – Compost manure @ 5 t/ha	67	8.86	2.58	1.93
F8 – Compost manure @ 5 t/ha + AMF	71	9.32	2.73	2.02
F9 – Compost manure @ 10 t/ha	68	8.98	2.68	1.95
F10 – Compost manure @ 10 t/ha + AMF	86	10.00	2.78	2.17
Mean	**73**	**9.36**	**2.69**	**2.02**

Table 9a: Fruit (tamar) yield of date palm as influenced by the sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait (average of 3 years).

Treatment	Tamar yield (kg/tree)	Tamar yield (kg/donum)	Tamar yield (tons/ha)	Increase over control(%)
F1 – Control	58.43	1192	11.92	-
F2 – Arbascular Mycorrhizal Fungi(AMF)	61.20	1249	12.49	4.30
F3 – Sewage sludge @ 5 t/ha	64.70	1320	13.20	10.07
F4 – Sewage sludge @ 5 t/ha + AMF	67.00	1376	13.76	14.34
F5 – Sewage sludge @ 10 t/ha	66.30	1353	13.53	12.82
F6– Sewage sludge @ 10 t/ha + AMF	71.97	1469	14.69	22.27
F7 – Compost manure @ 5 t/ha	63.73	1300	13.00	8.70
F8 – Compost manure @ 5 t/ha + AMF	65.53	1337	13.37	12.03
F9 – Compost manure @ 10 t/ha	64.67	1320	13.20	10.55
F10 – Compost manure @ 10 t/ha + AMF	70.00	1429	14.29	19.31
S.Em.+_	3.242	66.152	0.662	-
C.D. at 5%	9.102	185.722	1.857	-
Mean	**65.40**	**1335**	**13.55**	**-**

Table 9b: Actual soil moisture content(%) before irrigation at 30cm depth during flowering period of date palm as influenced by sources and levels of organic manure application in conjunction with arbascular mycorrhizal fungi(AMF) on sandy soils in Amghara Station of The State of Kuwait during 3 years from 2013-2016.

Treatments	Actual soil moisture content(%)			
	2013-14	**2014-15**	**2015-16**	**Mean**
F1 – Control	7.54	8.04	7.69	7.76
F2 – Arbascular Mycorrhizal Fungi(AMF)	7.98	9.48	9.91	9.12
F3 – Sewage sludge @ 5 t/ha	8.94	10.44	10.46	9.95
F4 – Sewage sludge @ 5 t/ha + AMF	11.42	12.92	13.03	12.46
F5 – Sewage sludge @ 10 t/ha	9.49	10.99	11.10	10.53
F6– Sewage sludge @ 10 t/ha + AMF	12.97	14.47	14.60	14.01
F7 – Compost manure @ 5 t/ha	8.49	9.99	9.96	9.48
F8 – Compost manure @ 5 t/ha + AMF	11.12	12.62	12.74	12.16
F9 – Compost manure @ 10 t/ha	9.17	10.67	11.98	10.61
F10 – Compost manure @ 10 t/ha + AMF	12.36	13.86	13.96	13.39
Mean	**9.95**	**11.35**	**11.54**	**10.95**

Treatment wise soil nutrient status at 0-25 cm and 25-50 cm depths after the harvest of tamar fruits from the date palm trees in the ICARDA experimental site during 2015-2016 at Amghara Station, Kuwait has been presented in Tables 10 & 11.

Table 10: Treatment wise soil nutrient status at 0-25 cm depth after the harvest of tamar fruits from the date palm trees in the ICARDA experimental site at the end of three years' project at Amghara Station, Kuwait.

Parameter	**Unit**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
1. pH	-	8.00	7.41	8.04	7.81	8.03	7.91	7.62	7.35	7.61	7.93
2. ECe	dS^{-m}	1.15	3.40	0.60	2.13	0.77	1.31	3.59	5.62	3.43	0.95
3. Ca	Meq/L	2.6	11.8	1.4	3.6	8.1	3.0	8.4	14.0	5.8	2.2
4. Mg	Meq/L	0.4	4.0	1.4	1.8	1.2	0.8	1.4	5.8	2.0	0.8
5. HCO_3	Meq/L	3.4	6.6	5.0	3.0	4.4	3.4	6.0	7.6	2.4	4.0
6. Cl^-	Meq/L	8	17	5	17	7	8	25	3.4	35	7
7. O.M	%	0.71	1.48	0.67	1.82	0.98	1.45	0.07	0.17	1.88	0.84
8. B	ppm	0.681	0.404	0.046	0.271	0.359	0.306	0.989	0.791	0.433	0.143
9. N	%	0.008	0.006	0.009	0.008	0.005	0.007	0.008	0.005	0.006	0.009
10. P_2O_5	ppm	25.4	59.2	37.4	80.4	41.8	27.8	31.2	45.2	64.487.56	36.6
11. Sand	%	82.64	81.28	83.28	78.56	82.28	81.6	82.64	83.28	87.56	87.92
12. Silt	%	14.36	13.72	12.72	15.44	13.72	12.44	13.36	12.72	12.44	11.08
13. Clay	%	3	5	4	6	4	6	4	4	0	1

Table 11: Treatment wise soil nutrient status at 25-50 cm depth after the harvest of tamar fruits from the date palm trees in the ICARDA experimental site at the end of three years' project at Amghara Station, Kuwait.

Parameter	**Unit**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
1. pH	-	7.91	7.65	8.00	7.83	7.93	7.84	7.82	8.08	7.41	7.91
2. ECe	dS^{-m}	5.17	5.44	0.84	1.18	1.16	1.48	5.84	1.07	7.36	4.99
3. Ca	Meq/L	1.4	10.8	2.2	2.4	3.2	3.0	10.8	2.1	13.0	12.0
4. Mg	Meq/L	0.7	2.6	2.4	0.8	3.0	2.6	4.0	1.8	3.0	4.4
5. HCO_3	Meq/L	3.6	6.0	4.0	3.8	3.8	2.6	4.0	3.0	5.6	5.2
6. Cl^-	Meq/L	28	39	6	8	10	10	34	7	6	35
7. O.M	%	0.47	0.94	0.30	0.57	0.81	1.31	0.57	0.07	1.72	0.81
8. B	ppm	0.514	0.662	0.737	0.206	0.163	0.454	0.269	0.244	0.712	0.469
9. N	%	0.038	0.008	0.005	0.007	0.008	0.005	0.011	0.010	0.007	0.005
10. P_2O_5	ppm	19.16	31.2	29.8	45.2	46.0	12.6	21.4	8.2	49.4	15.2
11. Sand	%	82.28	81.28	80.92	75.56	83.28	77.56	87.28	83.92	74.56	81.28
12. Silt	%	13.72	13.72	14.08	17.44	11.72	14.44	10.72	14.08	17.44	13.72
13. Clay	%	4	5	5	7	5	8	2	2	8	5

Figures

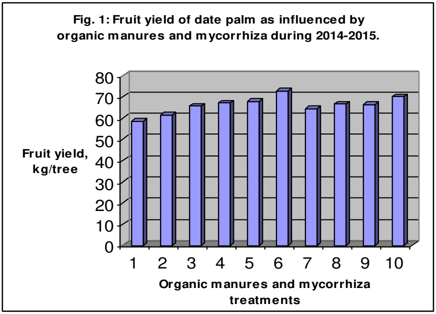


Fig. 1: Plan of layout of date palm trees selected for ICARDA project at Amghara (October, 2013-September, 2016)

Sl. No. of Rows	**Serial Number of Date palm Trees Selected for Experimentation**											
V	5	6	15	16	25	26	35	36	45	46		
IV	4	7	14	17	**X**	27	34	37	44	47		
III	3	8	√	18	23	28	33	38	**X**	**X**	**32**	**43**
II	2	9	12	19	22	29	√	39	42	49	**24**	**48**
I	1	10	11	20	21	30	31	40	41	50	**13**	**X**

↑
N

Note: Tree spacing: 7 m x 7 m = 49 m^2 **No. of bubblers/tree:** One

X No tree exists √ Tree exists, small & non-bearing(Not selected)

Fig. 2: Plan of layout of ICARDA project on the evaluation of date palm production and soil properties in relation to organic manure and mycorrhiza in Amghara area of The State of Kuwait.

R I		**R II**		**R III**		**R IV**		**R V**			
5 **F8**	**6** **F3**	**15** **F3**	**16** **F5**	**25** **F1**	**26** **F4**	**35** **F2**	**36** **F5**	**45** **F9**	**46** **F10**		
4 **F4**	**7** **F1**	**14** **F9**	**17** **F10**	**X**	**27** **F9**	**34** **F4**	**37** **F6**	**44** **F1**	**47** **F5**		
3 **F10**	**8** **F9**	√	**18** **F1**	**23** **F10**	**28** **F6**	**33** **F7**	**38** **F1**	**X**	**X**	**32** **F3**	**43** **F8**
2 **F2**	**9** **F5**	**12** **F7**	**19** **F2**	**22** **F2**	**29** **F8**	√	**39** **F10**	**42** **F6**	**49** **F7**	**24** **F5**	**48** **F2**
1 **F6**	**10** **F7**	**11** **F6**	**20** **F4**	**21** **F7**	**30** **F3**	**31** **F9**	**40** **F8**	**41** **F3**	**50** **F4**	**13** **F8**	**X**

↑

N

Note: Tree spacing: 7 m x 7 m = 49 m^2; **No. of bubblers/tree:** One

X No tree exists; **√** Tree exists, small & non-bearing (Not selected)

Plate 1: Mycorrhizal fungi & its association with plant & soil system

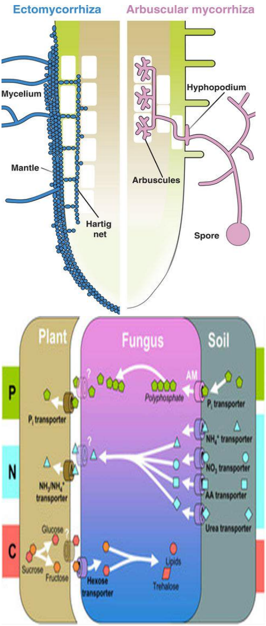


Fig. 1: Fruit yield of date palm as influenced by organic manures and mycorrhiza during 2013-2014.

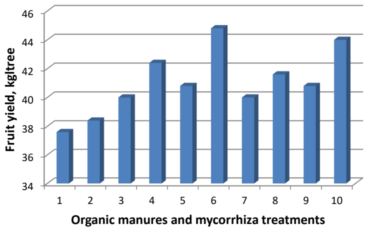


Fig. 1: Fruit yield of data palm as influenced by organic manures and mycorrhiza during 2015-2016.

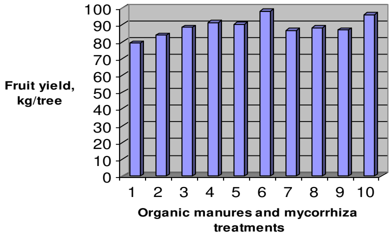
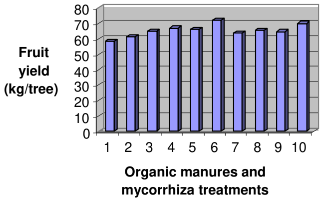


Fig. 1: Fruit yield of date palm as influenced by organic manures and mycorrhiza(average of 3 years).



Project development of sustainable date palm production systems in the GCC countries of the Arabian Peninsula: objectives, activities and major achievements

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Abstract

The project Development of Sustainable Date Palm Production systems in the GCC Countries of the Arabian Peninsula, is executed in the six GCC countries (Oman, UAE, KSA, Bahrain, Qatar and Kuwait) and financed by the GCC. The project aims to: Use of Proper agro-management techniques, Develop of proper IPM programs against pests and diseases, Development of proper post-harvest techniques to reduce losses, and improve marketing, Characterize and finger prints the major date palm local cultivars, Reinforce building of national programs in the area of date palm agro-management and Enhance Networking capabilities for the exchange of information, databases, services derived from the project activities. The project has 4 technical fields in problem-solving research component (Propagation and Crop Management, Integrated Pest Management, Postharvest and processing and Biotechnology and germoplasm conservation). In the Capacity Building component, the project provides specialized training to strength the national agricultural research systems. In the Technology transfer component the project facilitate the transfer and adoption of suitable technologies developed regionally and/or internationally. The major achievements of the project are in Field operations are: Applying liquid pollination to facilitate the operation and maintain good level of fruit set ; Selecting bio-pesticides for controlling infestation by borers and mites and identification of some natural enemies for the control of Lesser date Moth ; Ameliorating the quality of dried dates and reducing the loss and the drying time by using Polycarbonate houses) ; Selecting SSR markers to characterize 60 cultivars from GCC countries (10 major cv from each country).

Keywords: GCC, Date Palm, Competitive advantages, competitiveness indices, market share, revealed comparative advantage, trade balance index, dates marketing, Gulf cooperation council (GCC).

Competitive Advantage of GCC date palm sector in the International market: Market shares, revealed comparative advantages, and trade balance indexes

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Abstract

In the GCC countries, date-palm sector is strategically important for the economic, social and environmental development. Therefore, markets globalization has had a huge impact on the comparative advantages of date exports from the GCC countries, highlighting a new range of necessary determinants for competitiveness of these countries on the international market of date. The current study is conducted in the framework of the "Development of sustainable date palm production systems in the GCC countries of the Arabian Peninsula"; project funded by the Gulf Cooperation Council (GCC) and led by the International Center for Agricultural Research in the Dry Areas (ICARDA), and aims to provide updated estimates of competitiveness indicators of the GCC countries on the international market of dates palm.

The study starts by a summary description of updated figures concerning date's production, yields, consumption trends and patterns of the different GCC countries. This first descriptive part of the paper also include a presentation of the date trade matrix (destinations of exports and imports) of the considered countries. In a second part of this section, a set of competitiveness indicators were calculated to better reflect on the date trade balances performances of each of the GCC countries. The measures of competitiveness conducted in this paper include: i) the Market Share (MS); ii) the Revealed Comparative Advantage (RCA); and iii) the Trade balance Index (TBI). The Market share indicator was used to identify size advantages and the degree of specialization of a given country on the international market of a given commodity. The RCA has been defined as a measure of performance of international trade competitiveness of a given country for a given commodity. And finally, the TBI is used to analyze whether a country has specialization in export (as net-exporter) or in import (as net-importer) for a specific group of products. Data from both FAOSTAT and UN COMTRADE sources was used for the calculation of these indicators. Empirical findings shown that GCC and North African countries are holding more than 70% of the international market of dates. The sum of market shares of the 6 GCC countries was about 30% of the international date market during 2015. This is showing that these countries together have strong potential for dominating the international date market. In terms of growth, it was clear that all GCC countries, including the least present on the international market are progressing quite positively with increasing shares from one year to another. In terms of RCA, the highest RCA value was recorded for Saudi Arabia. The RCA index of Saudi Arabia was about 43.5 in 2013, indicating that the country date export share for 2013 is 43.5% higher than its share in total world export of agricultural goods. Finally, TBI results show the existence of structural differences between Saudi

Arabia and UAE in terms of dates export and import patterns. These two countries are both the main players in date export in the CC area. However, even though UAE is a net exporter of dates, its TBI is much lower than the TBI of Saudi Arabia, showing that UAE is also importing a higher proportion of its exported dates compared to Saudi Arabia. The date trade patterns among the GCC countries shows that there is a wide scope of coordination between the different trade strategies of these countries, through specialization and division of tasks. This can generate important opportunities for gaining more weight on the world market of dates.

Keywords: Competitive advantages, competitiveness indices, market share, revealed comparative advantage, trade balance index, dates marketing, Gulf cooperation council (GCC).

1. INTRODUCTION AND BACKGROUND

Date palm production is a strategic sector in most of the GCC countries. The sector is one of the oldest economic activity in the Arabian Peninsula and continue to play a key role in the culture of its population. In addition to the importance of dates for domestic consumption, the date sector is also a source of employment, income generation, and trade in many of these countries. The date fruit, known for its nutritive as well as spiritual value, is marketed all over the world under different forms (fresh fruits, or high value confectionery). In some very arid areas, date fruit remains as an important source of subsistence and resilience for local populations.

The share of the GCC countries in the total world export value was about 25% in 2013 (FAO, 2013). The largest exporting countries of date on the international market are Tunisia, Algeria, Egypt, Saudi Arabia, and United Arab Emirates. The high competitiveness on the international market of dates involves developing analytical reflections and studies on sources of competitiveness as well as its indicators, which can serve to set targets, objectives, and trade strategies. This competitiveness analysis and performances of the GCC countries on the international market of dates is the purpose of this study.

There is however no unique definition of the concept of competitiveness. Divergent approaches to competitiveness have produced different definitions. The concept is indeed very general and multifaceted and has a multidimensional nature linked to the optimal use of resources and geared to capturing development perspectives (Biggeri, 2007). With the increased globalization of the economy, the term competitiveness has become ubiquitous. The World Economic Forum's Global Competitiveness Report defines competitiveness as "the set of institutions, policies, and factors that determine the level of productivity of a country." An earlier definition by Michael Porter (1990) of the competitiveness of national (and regional) economic sectors and clusters of firms (at meso-level) refers to the competitiveness as the "ability of firms to achieve sustainable success against their competitors in other countries, regions or clusters". This is different from the definition of firms' competitiveness which is rather related to the "ability to provide products and services more effectively and efficiently than relevant competitors and to generate, at the same time, returns on investment for stakeholders (Porter 1990).

Lachaal (2001) provides a comprehensive assessment of what can be considered as determinants of competitiveness (see Fig 1). These include national (natural resources endowment, level of technology use, productivity of production within the cluster of firms of a given sector, scale economies, transport and marketing costs, etc.) and international (exchange rate, world market conditions, international transport costs: distance of countries from their relevant markets) determinants.

Biggeri (2007) considers that measures of competitiveness at economic sector level include the overall profitability of one nation's firms in the sector, the trade balance in the industry, the balance of outbound and inbound foreign direct investment, and direct measures of cost and quality at industry level. In line with this statement, Lachaal (2001) states that measures of the competitiveness may include different types of indicators such as:

- Measures related to the production costs (comparative advantages/relative costs/absolute costs, etc),
- Measures related to the factor productivity, and Measures of trade performances.

In this research paper, we rather focus on the last bullet by calculating a set of trade performance indicators for the GCC countries operating on the international date market. In the rest of this document, we present these indicators, as well as the way they are calculated and the source of data used for their collection.

2. Purpose of the study

The aim of this study is analyze the trade performances of GCC countries on the international market of dates. A list of performance indicators will be measured and reported. These include: i) the market share of a given country on the world export market of date, ii) the revealed comparative advantage, and iii) the trade balance index. The assessment and comparison of these indicators among the GCC countries, as well as with other major competitors at the international level would provide insights that can be used for setting improved trade strategies and market opportunities.

3. Methodological framework

Competitiveness is a complex concept which embed different indicators and involves different methods for their calculation. The competitiveness of a given sector (or subsector) can be analyzed from several perspectives (Han, Wen, & Kant, 2009). The focus of this paper is about the competitiveness of the date subsector in the GCC countries. After a quick overview of the trends of different production and trade aggregates, we provide a calculation of a list of indicators which are widely used in competitiveness studies. The set of chosen indicators includes the Market Share (MS), Revealed Comparative Advantage (RCA), and the Trade Competitiveness (TC).

3.1. Market Share (MS)

The Market share indicator is used to identify size advantages and the degree of specialization of a given country on the international market of a given commodity (Han et al., 2009). It is expressed as the percentage of the total available market of commodity *i* (market segment) which captured by a country *j*. MS is calculated as shown in the following equation.

= ⁄

Where is the export of commodity *i* of country *j*; and is the world export of commodity *i.*

3.2. Revealed Comparative Advantage (RCA)

The RCA has been defined by Balassa (1979) as a measure of international trade competitiveness of a given country for a given commodity. It is calculated as being a ratio of the export share of commodity j of a country I, compared to his total exportations (all sectors/commodities included), by the world export share of the same commodity compared to total world export (all sectors/commodities included). The RCA can be calculated as by the following equation.

∕
= ∑ ∑ ∕

∑ ∑

Where, X is the export of commodity *i* by country *j*; ∑ are total exports of country *j*; ∑ are total world exports of commodity *i;* and ∑ ∑ are the total world exports. If RCA > 1, then a comparative advantage is revealed; if RCA < 1 then a comparative disadvantage of the respective country is revealed.

3.3. Trade Balance Index (TBI)

Trade balance index (TBI). It is employed to analyze whether a country has specialization in export (as net-exporter) or in import (as net-importer) for a specific group of products. TBI was used as one of the crucial variables for analyzing the catching-up economies comparative advantage. The TBI value indicates a qualitative structure of product export and import and trade flows. It is formulated as follows:
= (−)⁄(+)

Where Xij and Mij represent exports and imports, respectively, of country i for product j. The TBI value varies between –100 (if a country only imports) and 100 (if a country only exports). Any value within –1 and +1 implies that the country exports and imports a commodity simultaneously. A country is referred to as "net importer" in a specific group of product where the value of TBI is negative and as "net exporter where the value of TBI is positive.

3.4. Source of data

UN COMTRADE1 and FAO2 annual time series (from 1961 to 2011) of all national aggregates including average yield, total country production, consumption, export, and import are the primary source of data used in this research. Data from both sources was continuously cross checked in order to be sure of its reliability.

4. RESULTS AND DISCUSSION

4.1. Production and consumption trends, and patterns of date palm in the GCC countries

Official FAO statistics (Table 1) show that dates areas, production and yield are progressing quit differently among the GCC countries. Saudi Arabia, Oman, and United Arab Emirates (UAE) have the highest harvested areas in 2014 with respectively 107 281 ha; 36 255 ha and 28 485 ha in the three countries. While this area was stable in Oman during the last two decades (from 35508 ha in 2000 to 36255 ha in 2014), it has rather been decreasing in both Saudi Arabia (from 142450 ha in 2000 to 107281 ha in 2014) and UAE (from 185330 ha in 2000 to 28485 ha in 2014). This decrease was the highest in UAE with more than 80% between 2000 and 2014.

Harvested areas in Bahrain, Kuwait and Qatar are still very limited with respective values of 3195 ha; 8931 ha; and 2290 ha during 2014. However, harvested areas in these three countries have been quickly progressing during the last two decade with an average annual increase of about 20%, 40%, and 1.38% in respectively Bahrain, Kuwait, and Qatar (see table 1).

The highest average yields (calculated over the period 2000-2014) are recorded in Qatar, Bahrain, and Kuwait with respectively 112006 Kg/ha; 86950 kg/ha; and 85173 Kg/ha (see figure 1). Average yields are the lowest in Saudi Arabia and UAE, with respectively 63105 Kg/ha and 49001 kg/ha in both countries. These yield values combined to the statistics on harvested areas makes Saudi Arabia the first producer of dates in the GCC region, with an average annual production3 of 933 899 tons per year, followed by UAE producing and average of 623 900 tons per year. More insight about the production levels (total domestic production quantities) of dates and their historical trends, including annual growth rates are given in the Figure 2. Calculated over the period 2000-2014.

1https://comtrade.un.org/data/
2http://www.fao.org/faostat/en/

In terms of consumption, the FAO figures show that Dates consumption per capita is the highest in Oman with a value of 68 Kg/capita/year, followed by Saudi Arabia with a value of 34 Kg/capita/year (see figure 3). However, consumption statistics on Qatar, and Bahrain are not available. The trends of dates consumption per capita in the considered countries is shown to be decreasing during the period 2000 to 2013.

Part of date production is also used as feed in some countries. This practice is not only observed in GCC countries, but is also frequent in other North African countries such as Tunisia. Another part of date production is wasted. Figure 4 shows some figures of dates quantities used as feed and wasted in the considered GCC countries. It shows that up to 38% of the dates production was served to animals as feed in UAE during 2013. For Oman and Tunisia, this figure is variable between 4% and 15%, among years. The volume of wasted dates is also important in the considered countries. The lowest wasted percentage (of production) is recorded in Saudi Arabia (1%), while a highest rate of 13% is recorded in Kuwait.

4.2. Date palm trade matrix of the GCC countries

The trade matrix of dates for the GCC countries is represented through a list of exported quantities from each of these countries to different destinations in the world. However, due to the high number of destinations, we only summarized in Table 2 the number of countries to which each of the respective GCC countries are exporting to. We also added the exported quantities of dates and their respective value, for each GCC country. Results in table 2 shows that Saudi Arabia and Emirates are the most active in terms of market diversification expressed by the number of countries to which they are exporting dates. In 2015, Emirates was exporting to 103 countries, while Saudi Arabia was exporting to 66 countries. Qatar and Oman are also exporting to high number of countries, with respectively 62 and 28 export destinations.

In addition to the figures in table 2, we wanted to stress the intensity of dates exchange, particularly among the GCC countries (see figure 5). Figures show again that Saudi Arabia and Emirates are the most active in terms of export on the GCC market, with high and growing export values to the different GCC countries. On the GCC market, UAE is mostly exporting to Oman, followed by Saudi Arabia and Qatar. While Saudi Arabia is mostly exporting to UAE followed by Kuwait and Qatar. Most of the Omani dates is also exported in the destination of UAE, which is showing that UAE is the first exporter and importer partner of Oman. This can be due to different consumers' preferences for dates in both countries and/or to different performances of dates processing companies in both countries.

4.3. Trade performances of dates: Results of indicators calculations

Market share indicator (MSI)

This section considers the GCC share in world export market of dates (trade between and beyond GCC economic area), and compares this share with the main competitors. The analysis shows that GCC and North African countries are holding more than 70% of the international market of dates. Figure 6 shows how this market is shared among the GCC countries and their direct competitors from North Africa, such as Tunisia, Algeria, and Egypt. In addition to Israel, who constantly holds around 10% of the international market of dates, Tunisia is dominating in terms of market share, with an average value of around 25% over the last decade. Algeria and Egypt are simultaneously holding around 4.2% and 3.8% of the market (Figure 6).

The sum of market shares of the 6 GCC countries was about 30% of the international date market during 2015. This is showing that these countries together have strong potential for dominating the dates market (see figure 6). This total share was constantly progressing during the last decade, from a total value of 22.98% in 2005 to 30.49% in 2015. This progress especially refers to the rapid increase of the shares of Saudi Arabia, UAE, and Oman, as shown in Figure 7.

Figure 8 is providing the values of market shares separately for the GCC countries. The reason is that the market shares of Bahrain, Kuwait, and Qatar are much smaller than these of Oman, UAE, and Saudi Arabia, in such a way that the presentation of all figures together might hide the small shares (values) of the least important countries in terms of market share.

In terms of progress, it is clear that all GCC countries, including the least present on the international market are progressing quite positively with increasing shares from one year to another. This is especially true for Qatar and Kuwait. Oman, Saudi Arabia, and UAE, also have the same trend with market shares increasing respectively from 0.34%, 8.65%, and 13.97% in 2005 to 1.14%, 12.95%, and 16.33% in 2015.

Based on these figures, it is clear that coordination between the different trade strategies of the GCC countries, through specialization and division of tasks, can generate important opportunities for gaining more weight on the world market of dates. Dates producers and producers organizations in major GCC producer countries, for instance, can interact regularly with dates processing and packaging manufactories and learn about exigencies and requirements on different markets. Processing manufacturers can also develop strong interactions with date producer's organization to ensure the alignment of production procedures with specific international markets standards and norms. This type of interactions among the professional organizations within the GCC countries can be mutually advantageous and self-reinforcing, but will not happen without effective coordination at the policy making levels.

"Revealed Comparative Advantage" of dates for the GCC countries (RCA)

The revealed comparative advantage is an index used in international economics for calculating the relative advantage or disadvantage of a certain country in a certain class of goods or services as evidenced by trade flows. It is based on the Ricardian comparative advantage concept, with the assumption that the commodity pattern of trade reflects inter-country differences in relative costs as well as in non-price factors.

The higher the RCA index value, the greater the importance of date relative to other agricultural exports. Thus, an RCA index of 43.5 for Saudi Arabia in 2013, would indicate that the country dates export share for 2013 is 43.5% higher than its share in total world export of agricultural goods.

Results of the RCA calculation, shown in table 3 are very close the RCA patterns identified by El-Habba Al-Mulhim (2013), with Kuwait and Bahrain having no RCA during the study period, and Saudi Arabia and Emirates having the highest comparative advantages among the GCC countries. Among competitors, Tunisia and Algeria are the countries with the highest RCA in terms of date trade.

In this study, we also used both FAOSTAT and UN COMTRADE data bases as some small differences exists among both in terms of quantities and values of date traded. Results in Table 3 below were computed from UN COMTRADE data, while the results showing the trends of RCA in each of the GCC countries were calculated from FAOSTAT data and are shown in Figure 9. Results from both data sources are converging and confirming the RCA patterns also identified by other studies such as El-Habba & Al-Mulhim (2013).

Results of "Trade Balance Index" (TBI)

The trade balance index is an indicator of countries specialization in either export or import of a given commodity. As argued in section 3.3, any TBI value comprised between -1 and +1 indicates that the country is both importing and exporting date on the international market. Negative values of TBI (as highlighted in table 10), refer to countries which are net importers while positive values refer to net exporter countries. Table 4 shows the existence of structural differences between Saudi Arabia and UAE in terms of dates export and import patterns. These two countries are both the main players in date export in the CC area. However, even though UAE is a net exporter of dates, its TBI is much lower than the TBI of Saudi Arabia, showing that UAE is also importing a higher proportion of its exported dates compared to Saudi Arabia. Saudi Arabia is a net exporter with very limited quantities of date importation. Most of its date export is actually driven by domestic production.

In 2013, Bahrain, Kuwait, and Qatar were recorded as net importers of date, with most of their domestic consumption coming from importations. In 2013, the TBI values for these countries were respectively about -0.97, -0.91, and -1.

5. Concluding remarks and implications

The present study was mainly focusing on analyzing the trade performances of GCC countries on the international market of dates. A list of performance indicators, including the Market shares, revealed comparative advantage, and trade balance index, have been be measured. The assessment and comparison of these indicators among the considered countries can be used for setting improved trade strategies and access to more valuable market opportunities.

Results of our analysis have shown that the market share of the 6 GCC countries considered was about 30% of the international date market during 2015. This is showing that these countries together have strong potential for dominating the international date market. In terms of RCA, the highest RCA value was recorded for Saudi Arabia, showing that the country dates export share for 2013 is 43.5% higher than its share in total world export of agricultural goods. Moreover, Saudi Arabia had a positive trade balance, showing that this country is the only net exporter of date in the region. Despite the high RCA of UAE, its TBI was lower than Saudi Arabia showing that UAE is also importing a higher proportion of its exported dates.

The date trade patterns among the GCC countries shows that there is a wide scope of coordination between the different trade strategies of these countries, through specialization and division of tasks. This can generate important opportunities for gaining more weight on the world market of dates. Dates producers and producer's organizations in major GCC producer countries, for instance, can interact regularly with dates processing and packaging manufactories and learn about exigencies and requirements on different markets. Processing manufacturers can also develop strong interactions with date producers' organizations to ensure the alignment of production procedures with specific international markets standards and norms.

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Declarations of conflict of interest
The authors report no declarations of conflict of interest.

REFERENCES

Balassa, Bela, 1979, "The Changing Pattern of Comparative Advantage in Manufactured Goods", Review of Economics and Statistics, 61 (2) May: 259-266.

Biggeri, L., (2007)."New challenges in the measurement of competitiveness in economic globalization".

En: the ESS response to globalization - are we doing enough? Budapest.

El-Habba M.S and Al-Mulhim, F., 2013. The competitiveness of the Saudi Arabian date palm: An analytical study. African Journal of Agricultural Research. Vol. 8(43), pp. 5260-5267.

FAO, 2013. Food and Agriculture Organization online dataset. http://www.fao.org/faostat/en/ (Last accessed: April 2017).

Han, X., Wen, Y., & Kant, S. (2009). The global competitiveness of the Chinese wooden furniture industry. Forest Policy and Economics, 11(8), 561–569. https://doi.org/10.1016/j.forpol.2009.07.006

Lachaal L, 2001. La competitivité : Concepts, définitions et applications. In in Laajimi A. (ed.), Arfa L. (ed.). Le futur des échanges agro-alimentaires dans le bassin méditerranéen : Les enjeux de la mondialisation et les défis de la compétitivité. Cahiers Options Méditerranéennes; n. 57, 29-36.

Michael E. Porter, 1990. "The Competitive Advantage of Nations," Harvard Business Review, March 1990, http:// hbr.org/1990/03/the-competitive-advantage-of-nations/ar/1.

Tables

Table 1. Date areas, production and yield of the GCC countries

GCC Countries	Items	Units	2000	2005	2010	2014
Bahrain	Area harvested	ha	823	1400	1588	3195
	Production	tons	16508	12000	12472	11164
	Yield	kg/ha	200583	85714	78527	34937
Kuwait	Area harvested	ha	1350	2000	5089	8931
	Production	tons	10155	15800	32561	115213
	Yield	kg/ha	75222	79000	63977	129004
Oman	Area harvested	ha	35508	31353	31353	36255
	Production	tons	280030	247331	276405	328392
	Yield	kg/ha	78864	78887	88159	90578
Qatar	Area harvested	ha	1931	1444	2469	2290
	Production	tons	16116	19844	21491	27482
	Yield	kg/ha	83459	137424	87043	120009
Saudi Arabia	Area harvested	ha	142450	150744	155118	107281
	Production	tons	734844	970488	991546	766800
	Yield	kg/ha	51586	64380	63922	71476
United Arab Emirates	Area harvested	ha	185330	185330	197400	28485
	Production	tons	757601	757601	825300	255182
	Yield	kg/ha	40878	40878	41809	89586

Source: FAOSTAT (several years).

Table 2. Number of dates exporting markets for each of the GCC countries

	2012			2015		
	Number of countries	**Exported quantities (Tons)**	**Value of export (1000 US$)**	**Number of countries**	**Exported quantities (Tons)**	**Value of export (1000 US$)**
Oman	23	5814.9	7745.6	28	9141.6	11981.5
Bahrain	2	43.8	33.1	4	10.0	16.5
Kuwait	19	363.9	337.7	18	564.4	397.2
Qatar	n.a	n.a	n.a	62	534.8	351.4
Saudi Arabia	60	64299.0	74859.5	66	120358.0	136263.6
UAE	98	304090.5	129177.2	103	309782.1	171897.1

Source: UN COMTRADE database; (na: not available). Number of countries shows the number of countries to which each of the respective GCC countries is exporting dates; Exported quantities are expressed in Tons; Value of export is expressed in 1000 US$. Note: n.a: not available.

Table 3. Revealed comparative advantage of date trade for the GCC countries and their competitors

Countries	2002	2005	2007	2010	2013
DZA-Algeria	672.79	384.06	436.61	318.33	110.97
BHR – Bahrain	1.51	0.37	0.94	0.36	0.06
ISR – Israel	32.04	60.66	44.62	54.06	78.38
JOR – Jordan	4.97	6.30	6.83	7.71	7.08
KWT – Kuwait	4.09	na	0.73	1.71	1.04
OMN – Oman	9.71	5.39	22.67	17.29	12.98
QAT – Qatar	1.48	3.17	2.94	na	na
SAU – Saudi Arabia	104.68	45.11	31.67	47.13	43.51
UAE – Emirates	na	37.54	40.35	na	45.10
TUN – Tunisia	463.26	199.43	227.93	323.80	228.73
EGY – Egypt	7.23	4.02	3.43	14.67	10.19

Source: Calculations from UN COMTRADE dataset (several years).

Note: n.a: not available.

Table 4. Trade Balance Index of dates for the GCC countries and their competitors

Countries	2001	2005	2008	2010	2013
DZA-Algeria	1	1	1	1	1
BHR – Bahrain	-0.83	-0.95	-0.67	-0.90	-0.97
ISR – Israel	1	1	1	1	1
JOR – Jordan	-0.72	-0.50	-0.40	-0.41	-0.44
KWT – Kuwait	-1.00		-0.99	-0.95	-0.91
OMN – Oman	0.98	-0.32	-0.60	-0.18	0.01
QAT – Qatar	-1	-0.95	-1	-1	-1
SAU – Saudi Arabia	1	0.90	0.83	0.96	0.95
UAE – Emirates		0.17	0.05		0.14
TUN – Tunisia	1	1	1	0.99	0.99
EGY – Egypt	-0.04	0.77	0.55	0.84	0.68

Note: Positive values indicate that the country is a net exporter. Negative values indicate the country is a net importer.

Figures

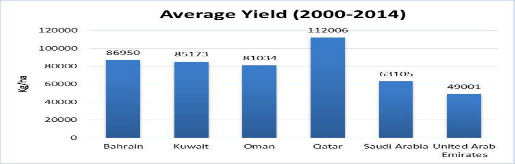

Figure 1. Average yield of dates in GCC countries calculated over the period 2000-2014

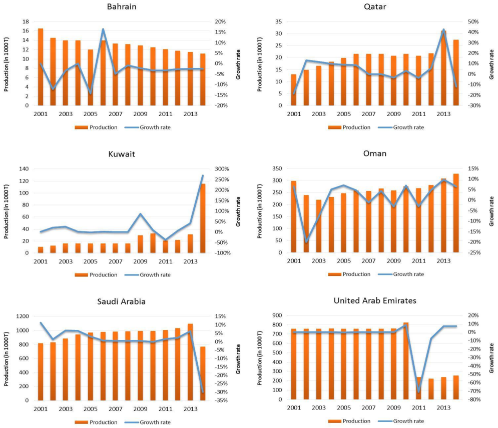

Figure 2. Production trends of dates in GCC countries

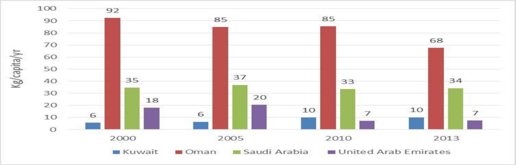

Figure 3. Trend of date consumption per capita, year (in Kg), 2013 data

Figure 4. Quantities and percentages of wasted dates and dates used as feed for selected countries

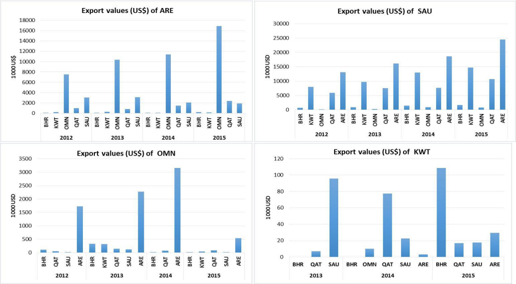

Figure 5. Export trade of dates among the GCC countries (UAE: United Arab Emirates, SAU: Saudi Arabia; OMN: Oman; and KWT: Kuwait) (Source: COMTRADE UN database)

Figure 6. Market share of selected dates exporting countries, including GCC countries and their competitors

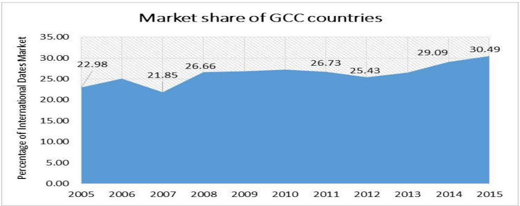

Figure 7. Trend of the GCC market share (sum of Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and Emirates) during the last decade (2005-2015)

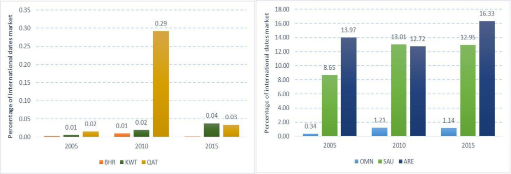

Figure 8. Market share of GCC countries on the international date market

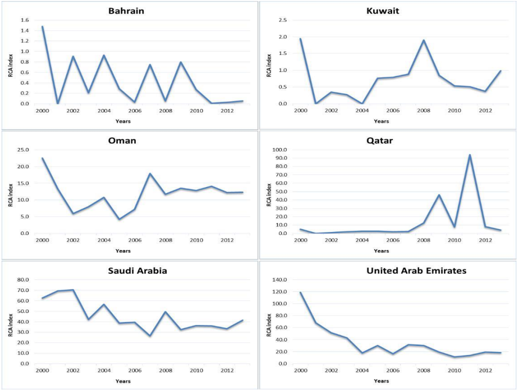

Figure 9. Trend of the Revealed comparative advantage of date trade for the considered GCC countries (for the period 2000 - 2013) source: Calculations from FAO dataset

Performance evaluation of an off-road light aerial platform for date palm cultivation

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Abstract

Date palm (*Phoenix dactylifera* L.) cultivation is characterized by several operations performed at the frond level. Fronds can be many meters above the ground, especially in older groves or plantations. Mechanization in date palm farms is still lacking or inadequate, especially in medium and small farms of non-industrialized countries, and operations at the frond level are still done manually by climbing up the tree. Working at height without specific equipment is difficult, tiring and risky and many accidents occur to workers when climbing on taller palms with the traditional belt-based climbing system. In large specialized plantations of valuable date varieties, aerial platforms are used, generally derived from the construction industry, with or without adaptations to the specific task. Nevertheless, the high purchase and maintenance costs don't allow for their use in smaller farms.

However even medium sized groves, where high value varieties are cultivated such as the world renowned Medjool in the Jordan Valley (H.K. of Jordan), could benefit of specialized mechanized equipment if of adequate size and cost, but suitable solutions have been missing until now.

With the aim of proposing a versatile machine for aerial operations in date palm medium-sized farms, in 2016 the Italian manufacturers CO.ME.T. and ERREPPI marketed a compact aerial platform mounted on an off-road light carrier, specifically designed for use in palm plantations.

The objective of this study is the evaluation of this self-moving aerial platform, named Xiraffe, in terms of timing, effectiveness and general attitude to work along the date palm cultivation process. This analysis is based on observations done and data collected in 2017, during harvesting field trials on Medjool date palms in the Jordan Valley. These trials, carried out on palms of different height and characteristics, aimed at comparing mechanized and traditional manual harvesting, which is still the most common method in the study area. The results showed that this small sized and agile machine proofs to be effective while capable of improving work safety and timing when used to harvest palms between 6.0 and 9.4 meters high. However, the manual harvest is still more effective for medium and small farms in the test environment, but some technical improvement to the platform, such as modifying the bucket shape or providing it with specific tools for other operations (e.g. pruning, bagging or pollination), can reduce the gap, opening a completely new scenario in date palm cultivation.

Keywords: Medjool dates, Jordan, agricultural mechanization, harvesting, lifting of operators.

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is considered one of the most valuable and important fruit crop in its main distribution area of the Middle East and North Africa. It is among the very few plants that can thrive in arid environments and can provide significant resources for local populations (Chao and Krueger, 2007). More recently, because of the characteristics of the fruits that are appreciated in all the world, it was introduced in new areas such as America, Australia, Namibia, etc. (Garbati Pegna, 2008). In the last few decades, date production has grown extraordinarily and is expected to continue to raise (Chao and Krueger, 2007). Worldwide it has increased from 6,440,583 t in 2000 to 8,460,443 t in 2016, while the harvested area has expanded from 1,051,482 ha in 2000 to 1,353,159 ha in 2016 (FAOSTAT, 2018). This positive trend is also forcing date cultivation to develop new solutions to face the modern production challenges: timeliness of agricultural practices, increasing labor costs, scarcity of skilled labor, fatigue and risks inherent in this work are part of the main issues to address. In fact, date palm cultivation is characterized by several operations performed at the frond level which is often at more than 6 m above the ground and can reach up to 20 m in old plantations. In most plantations access to the frond level is still performed in traditional ways, where workers have to climb up the trunk with the help of belts or straps or long ladders or of other people piled up on each other's shoulders (Opara, 2003 in Garbati Pegna, 2008, Nourani, 2016). This makes these operations, among which harvest is obviously the most important, very difficult and risky, especially when palm height is over 6-8 m, causing many victims yearly or the abandoning of the higher palms (Garbati Pegna, 2008).

A major change that has occurred in date cultivation in the United States has been the mechanization of some cultivation practices and in particular the timing and method of harvesting (Barreveld, 1993 in Akyurt, 2002). During 1940's and 1950's, under the impact of increasing labor costs and ever-increasing height of the palms, some growers in the U.S. built large tractor-pulled harvesting towers, to avoid the need for ladders (Akyurt, 2002); starting from 1960, the use of truck-mounted hydraulic crane-like man-positioning machines was experimented, in order to move workers from palm to palm (Akyurt, 2002). Even if none of these attempts provided a significant increase in workers' productivity, the scarcity of labor was such that by 1966, 80% of the date crop in the US was being harvested with the use of these mechanical devices (Brown, 1983 in Akyurt, 2002). Nowadays other machines, which can harvest dates by shaking the plant or that by a slider mechanism and a grip force on the stem can climb up the operator to the frond, have been developed but these devices are not suitable for most date varieties and they still need many improvements (Shamsi, 1998). One of the most important steps forward in mechanizing operators' access to the fronds, flowers and fruits of date palms has been a sizable "U" shaped aerial platform, hold by a hydraulically moved telescopic boom or forklift mounted on an off-road carrier, that can provide support and space to several workers allowing them to work on a palm at the same time. This system, which offers good levels of efficiency and safety for workers and allows a faster repayment of the investment, is widely used in large specialized plantations where valuable varieties are cultivated. However, the high purchase and maintenance costs of this equipment make it not affordable for the medium and small farms (Garbati, 2008 and Shamsi, 1998). Further limits may be identified in difficulties of maneuvering in tight spaces caused by irrigation systems, an irregular layout of palms, insufficient tree spacing and intercropping (Shamsi, 1998). In some of these cases, or where different equipment is used, operators have to jump out from the platform to reach the fruits if the bunches are hidden by the fronds hence nullifying the safety aspect. Smaller elevating devices have also been proposed by various manufacturers (Garbati Pegna *et al*, 2012) but none has succeeded in capturing farmers' confidence.

Aiming at addressing the mechanization problems of smaller or difficult farms, by providing a flexible and light machine for operator's access to the high parts of the palms, two Italian companies ERREPPI and CO.ME.T. developed Xiraffe, an off-road light aerial platform, easily adaptable to diverse operating conditions, ensuring safety and easiness for working even at considerable heights. This machine constitutes a novelty in this sector for its characteristics and its suitability to work in harsh conditions and could represent a rentable solution, also susceptible of further developments, for field operations at frond level, offering suitable and affordable specialized mechanization solution even to the medium and small sized farms.

This study analysis the performances of Xiraffe focusing on the harvesting operations; the investigation is based on data collected in 2017, during field trials in Medjool plantations in Jordan Valley (H.K. of Jordan); these trials, carried out on palms of different height and characteristics, aimed at comparing mechanized and traditional manual harvesting, which is still the most common method in the study area.

2. Equipment

Xiraffe is a compact aerial platform, consisting of an articulated hydraulic boom lift mounted on a 4 wheel drive power unit, produced by a joint venture between ERREPPI, an Italian agro-transport vehicles producer, and CO.ME.T., an Italian lifting machines manufacturer.

2.1 The platform

The aerial part is composed of a basket held by an articulated boom lift, supported by four hydraulic outriggers. The platform lifting system is hydraulic and is activated by an always running hydraulic pump, moved by the power unit engine through a transmission belt. A main valve controls the hydraulic circuit, allowing fluid flow towards the lifting system only when the outriggers are well opened and the pressure on each one of them is between 5 and 295 kPa; at the same time, this valve doesn't allow the fluid to activate the outriggers when the boom has been moved from the initial position, in order to maintain the previously achieved stability. This system can only be interrupted by special emergency levers. The outriggers have a supporting surface of 314 cm^2 each and are controlled by a micro-switch based system and a warning signal is emitted when one of them is losing pressure on the ground and stability could be affected; furthermore, if the platform is subject to an excessive force further extension of the boom is disabled. The outriggers are positioned manually by the operator and the machine's attitude is checked on the control panel; the outriggers can adjust a maximum difference in height of 0.78 m, allowing the placing of the machine even on a very uneven terrain.

The aerial system can be guided by two fully hydraulic controls, one in the basket and the other at the base of the unit. The basket has a rectangular base of 1 m x 0.7 m and a height of 1.1 m with a rated maximum loading capacity of 150 kg, though no specific sensor controls this limit. The basket can be raised in 40 s at its maximum height of 9.8 m (basket floor) that means an average reach of 11.8 m, considering operator's height; the maximum outreach is 4.5 m from the boom's pivot vertical axis, that can be accomplished at a height of 7 m (Figure 2.1). The turret rotation range is 360° which makes it possible to access all the surrounding area. If safety limitations are respected the platform is rated for working up to a maximum wind speed of 15 m/s. The basket is provided with a 230 V electric outlet and a compressed air outlet for connecting different tools such as scissors, chainsaw, sprayers etc. At the moment either electricity or compressed air have to be provided by an external source. External canisters can be also hung on the basket's railing to collect the harvested dates.
Figure 2.2 shows the main dimensions of Xiraffe and its platform components.

2.2 The vehicle

The power unit is a compact all-terrain vehicle powered by a Yanmar L100N single cylinder, 435 air cooled Diesel engine, with a maximum power output of 8.3 kW. The engine is equipped with electric start and a battery of 50 Ah. The unit is 3.75 m long and 1.85 m wide

(Figure 2.2) with a front and rear wheel track of 1 m and 1.17 m respectively and a wheelbase of 1.975 m; the steering angle reaches 27.5° for each side; the ground clearance is 0.27 m and the total weight is 1,680 kg including the platform.

A synchromesh five gear plus reverse transmission allows Xiraffe to reach a maximum speed of 18.9 km/h while the average fuel consumption is about 0.7 l/h at an engine speed of 3,000 rpm. Disk brakes are mounted on each wheel. Transmission is part-time type, allowing to select traction on 2 or 4 wheels depending on the situation.

Being the platform load mainly concentrated on the back wheels, the total maximum weight per wheel reaches 565 kg, so low pressure flotation tires (82 kPa) have been adopted in order to allow moving also on soft or sandy soils without damaging the irrigation systems or the grove environment. The vehicle is also provided with a rear hitch for towing a 500 kg trailer.

These characteristics make the unit very versatile and well suited for operating also in tight and rugged environments.

3. Evaluation method

The evaluation was conducted by comparing manual and mechanically assisted harvesting in terms of time, productivity and out-of-pocket costs (fuel).

The tests were carried out in three different farms, all located within 1 km along the Middle Jordan Valley, in the municipality of Ma'addi, Al-Balqa governorate. In all farms, the main product was represented by Medjool dates with some secondary production such as Barhi dates, citrus and grapes. All farms were characterized by medium texture sandy/loamy, deep soils. All the cultivation practices carried out at the frond level, from pruning to harvesting, were still done manually, in the traditional way, while some mechanized equipment was used for the post-harvest processes. Tending of the palms was mainly managed by Egyptian workers, which are employed for the harvesting season or all year round.

Since most of the palms in all the farms were less than 15 years old, it was difficult to find plants that were sufficiently tall to make trials significative.

The farms were:

- *Al-Sughaiyer Co*. plantation, a 5.2 ha farm leased with a 5-year contract; the grove was constituted by 717 palms (700 Medjool and 17 Barhi) with an 8 x 8 m plant spacing. Pruning is done every second year, with a consequent presence of a high amount of leaves.
- *Jeneidi* farm, a family-run grove of 3.7 ha with 470 palms (455 Medjool and 15 Barhi) with a 9 x 9 m plant spacing. Secondary production is based on grapes and a nursery of ornamental palms is also part of the business. In this farm it has not been possible to find plants higher than 5.4 m because of the young age of the plantation, that was less than 12 years old.
- *Arar* farm owned by an entrepreneur with a 30 ha total area and 2,700 Medjool palms with an 8 x 8 m pattern. Other cultivations are Barhi (1,100 palms), citrus and grapes. In this farm there is a wide variety of palm sizes and shapes which allowed to carry out trials in many different situations.

The working procedure was the following:

Mechanically assisted harvesting

The first operation to be done, once the machine has reached the palm, is the positioning of the four outriggers; after this, the platform can be lifted by the operator itself or by a ground assistant. Once reached the cluster, the dates are collected and placed in plastic boxes and subsequently stored inside the canister. As common in Medjool plantations, only the ripe fruits are harvested and not the whole bunch: this is done by covering the bunch with a net bag and lightly shaking it, collecting only the dates that fall. Then, when the canister is full or all of the planned bunches have been harvested, the platform is lowered and the boxes manually passed by the operator on the basket to the one on the ground, which makes a first check and selection of the harvested dates. Once completed the task, the operator gets off from the basket, the outriggers are lifted and the machine is moved to the next position. At the end of each day, the fuel tank is filled up to monitor the daily fuel consumption.

Servicing date palms with a platform can be done in two different ways: the first is harvesting with the "360°" method, that means servicing a whole palm with just one positioning of the machine which is done near to the palm base (Figure 3.1). The second one is called "180°+180°" method that means servicing two half palms at a time and is done by placing the machine between two palms, in the middle of the row, and reaching only the half frond facing the machine (Figure 3.2).

In the Al-Sughaiyer Co. plantation, the selected palms were 15 years old and a total of 3 trials were done in the same day, using two times the "360°" method and one time the "180°+180°".

In the Jeneidi farm, 5 palms were harvested with the use of the Xiraffe; only the "360°" method was adopted, but the number of operators varied: the first three times employing only one skilled operator for all the activities, while the other two times two operators were engaged.

In the Arar farm, a total of 15 trials were done, 5 per day. In the first day the "180°+180°" method was used, while in the second and third days the "360°" technique was applied.

Changing servicing method and number of operators was a consequence of the novelty constituted by the kind of operation that needed to be gradually adjusted, since neither the Xiraffe nor the mechanically assisted harvest had been experimented before in these farms, and of the need to adapt to different situations that arise in the various locations.

Manual harvesting

The traditional manual harvesting was done by skilled operators climbing up the palms with or without the use of a belt, sometimes with the help of a ladder, and by placing the collected fruits in a small bucket, with a capacity of about 5 kg of Medjool dates, and lowering it to the ground with the use of a rope.

Data collected

The positioning of each harvested tree was detected by the use of the I-Phone 6 inbuilt GPS and the "GPS & UTM" application for I-Phone.

The distances from the trunk and the height of the dates bunches from the ground were measured with a Stanley TLM 99 laser telemeter, while the trunk circumferences with the use of a tape measure. The weight of harvested dates was measured by a farm's field spring scale, provided with a tray with a maximum capacity of about 3 kg of Medjool dates. These measurements were randomly verified in the warehouse by the use of a lager, 10 kg capacity, spring scale which evidenced an approximation of 10% in the field weighting system. The number of leaves of each palm was counted in order to assess the density and three density classes were defined: 120 leaves (high density, clusters completely covered by the frond), 100 leaves (medium density, clusters are still inside the frond but is much easier to reach them) and 90 leaves (low density, few clusters are out from the frond and all of them are easily reachable).

The daily volume of Diesel fuel consumption was quantified with a 2 l graduated container with a 0.25 l accuracy.

The time for carrying out the different operations in the manual and mechanically assisted harvesting was measured by the use of a chronometer.

& *Transfer*: in mechanically assisted harvesting the time needed to move the machine from palm to palm, from when the operator sits on the driver's seat to when he first touches the controls for the positioning of the outriggers. In manual harvesting, transfer is the time used for moving the equipment from one palm to the other. This was not always possible to measure so, after the first measurements, an average value of 30 s was considered for all the methods.

& *Placing*: the period from the end of transfer to the moment the operator is in the basket and ready for lifting. In this time also a 40 s period for loading the empty boxes into the basket is considered. In manual harvesting, placement is the preparation phase before climbing, during which the operator checks the palm and connects the rope to his arms or trousers' buckle. After the first measurements, it has been estimated in 30 s for the manual method.

& *Lifting*: from the first touching of the aerial platform control panel to the reaching of the date clusters. In manual harvesting, it is the period between the first touching of the ladder or of the plant to the touching of the clusters.

& *Harvesting*: the whole time used for harvesting, from the first touching of the cluster to the closing of the last cluster net bag. This is the same in both manual and mechanical one.

& *Descending:* from the completion of the harvesting to the positioning of the basket at the initial resting position. In manual harvesting is the time from the end of harvesting to the touching of the soil by the operator.

& *Unloading*: the time needed by the operator on the basket to hand over the boxes to the ground operator. In a few cases, because of the high amount of dates harvested, lifting, harvesting and descending operations had to be repeated twice.

& *Disengaging*: from when the operator steps out of the basket to when he sits again on the driver's seat after lifting the outriggers, ready for the transfer to the next palm.

When new equipment has to be used operators must be trained to learn how to manage and operate the machine properly and safely. In this case, due to lack of workers, only one operator from Jabaly Agricultural Co., a local Company which gave an important sustain to this study, could be trained and therefore was in charge of operating the machine during all trials. In this situation a 4-hour course, given by expert technicians of the manufacturing Companies, was enough to demonstrate and analyze all the different functionalities of Xiraffe, allowing the operator to experiment the different situations that may occur when using this kind of machine.

After this, the operator practiced for 3 days in order to acquire the necessary experience and skills.

During field trials, the operator was supported by a worker from the hosting farms. As a matter of fact, two workers are needed for the most efficient use of the platform: one in the basket for harvesting and one on the ground for assistance. Normally only the operator in the basket drives the machine and therefore needs specialized skills but, during the trials, the farm owners insisted to have their man harvesting the dates so the trained operator had to drive the machine from the ground panel, leaving to the other worker the task of harvesting and managing the dates. This system poses some hazard and should not be normally adopted, being even forbidden in many Countries. The weight of the operator in the basket was about 65 kg.

4. RESULTS AND DISCUSSION

The easiness of access to the date clusters depends mainly on their position, being frond coverage and height from the ground the most important factors.

Figure 4.1 shows how the machine proofed to be fully efficient in reaching clusters in a range between 4.4 and 9.4 m height with a normal frond density of about 100 leaves per plant.

In these conditions, Xiraffe allows harvesting all the palm's bunches with only one positioning ("360°" method). However, when cluster height is lower than 6 m, the boom geometry makes the platform progressively more difficult to manage because of the projection of the lower sections and the possibility of contact with the outriggers or other parts of the machine. On the other hand, when height is above 9.4 m, the accessible area decreases not allowing a 360° access around of the stem (Figure 2.1).

Figure 4.1 also shows how high leaf density negatively affects the access to the bunches; this is because the volume of the basket, hinders its capacity to penetrate through the canopy and the operator has to open his way between the leaves or crouch down in the basket, hence losing the full control of the platform. This problem is worsened by the canister that increase the volume of the basket and consequently its capacity to move across the fronds.

Concerning the stem's size, the results were not influenced by different diameters which ranged from 0.42 to 0.62 m, though keeping the stem trimmed is still recommended.

Out of 23 trials, only the last 8 were in the ideal height range and the team had reached complete confidence in operating the Xiraffe: figure 4.2 shows the total harvesting time spent during these 8 trials.

The average time for harvesting one palm was of 1,933 s (32'13") with two operators involved. However, harvesting time varied greatly from one palm to another being influenced by the different frond characteristics and amount of dates to be harvested. This is because when the total amount of dates per palm exceeds 60 kg, which is the maximum storage capacity of the basket and the canister together, an intermediate unloading is necessary, increasing the total time of the session. As a matter of fact, this extra operation nullifies the gain in productivity (quantity harvested/time), due to the high amount harvested in one single session, and builds up extra time losses.

Given that, during the 8 trials time increased with the amount harvested, a considerable difference can be noted between sessions where the total harvested amount was below or above 60 kg. In particular, sessions 5 was the slowest one because of the high amount of leaves on the palm that forced to remove the canister, hence reducing the storage capacity and making 2 extra unloading operations necessary.

This shows that, apart from the previously mentioned usefulness of pruning the older and less productive leaves, the size and shape of the basket are very important and solutions should be thought of for increasing its' loading capacity. In particular, the canisters didn't turn out to be a satisfying solution and should be redesigned. Also unloading of dates from the basket should be better organized since this was the slowest operation besides harvesting, taking 13.19% of the total time as shown in figure 4.3.

Among the other operations, lifting and descending were mainly related to the height of the palm and the ease of reaching the clusters or penetrating into the canopy. Disengaging was simple and fast as the transfer, which was mainly influenced by the distance between the plants that were not always near to each other. The placing was influenced by the terrain conditions and not always fast, but sped up with the operator's experience.

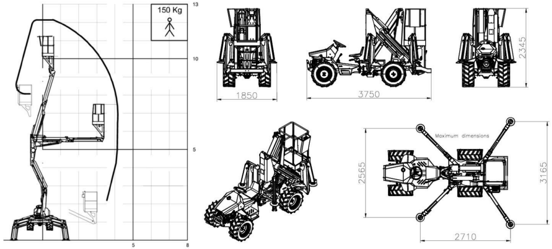


Figure 2.1 and 2.2. Platform aerial movements, on the left, and Xiraffe dimensions, on the right.

Figure 3.1 and 3.2. Placing for the two different methods: "360°", on the left, and "180°+180°" on the right.

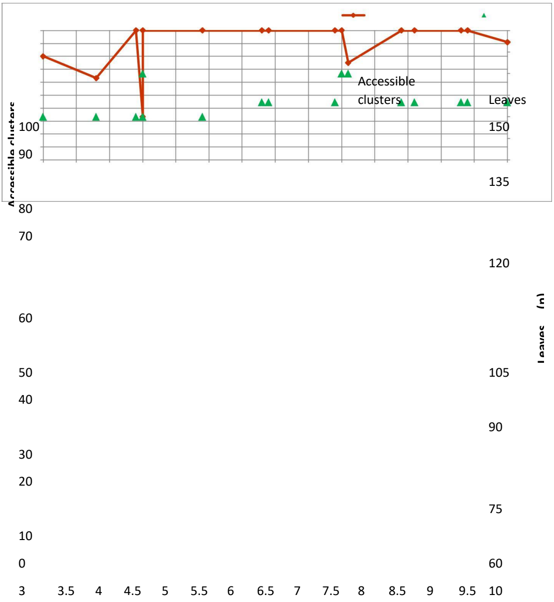


Figure 4.1. Main factors limiting access to the clusters in the 16 more significant trials.

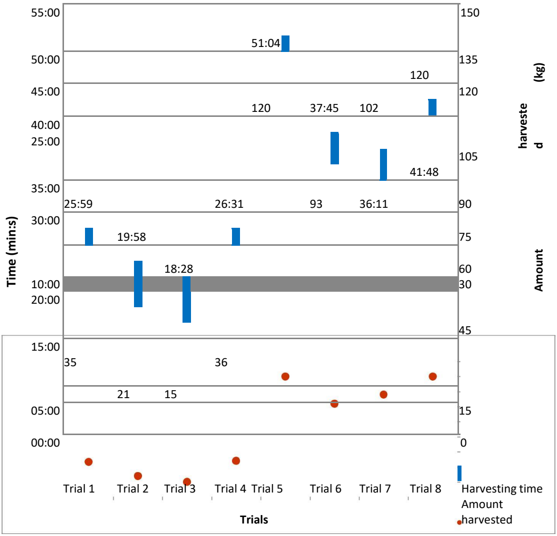
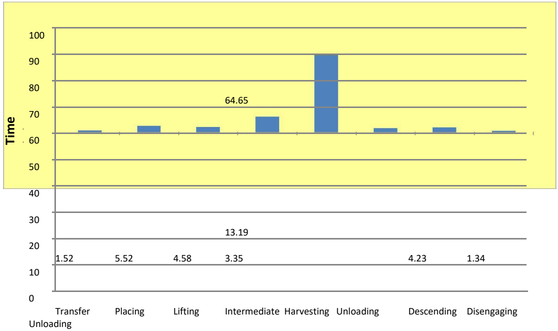


Figure 4.2. Harvesting time and quantity in 8 different trials.



Time sharing between the different operations

Figure 4.3. Time for single operations. Only trials where one intermediate unloading was needed have been considered.

GAP and GHP analysis in date small farms in Baharia oases

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Abstract

This paper aimed at assessing the agricultural practices related to food safety in small date farms in Baharia oases in terms of the food safety hazards biologically, chemically and physically that negatively affect consumers health due to poor agricultural practices in Small Date Farms (SDF). A questionnaire was designed to assess the agricultural practices in Baharia small farms in specific against the applicable requirements of the United States Department of Agriculture (USDA) Good Agricultural Practices (GAP) and Good Handling Practices (GHP) guide. The GAP and GHP practices in 38 date small farms located at Bawity, Mandisha and Zabo areas was evaluated. The descriptive approach was utilized, and some statistical indicators was extracted using the Microsoft excel program, i.e. average, minimum, maximum, standard deviation and variation coefficient. The study reached some major findings, which are: The average dilution of pesticides used in Baharia small farms is estimated at 0.01% (the standard dilution is 0.003%) with a minimum of 0.001 and a maximum of 0.05 with a variation coefficient estimated at 138%,This may attribute to unstandardized manners of dilution. also the pesticide per acre amounts are estimated in average at 2.649 litter (the standard amounts are 1 litter per acre) with a minimum of 0.571 litter and a maximum of 2.649 litters with a minimum of 0.571 litter and a maximum of 2.649 litters, with a variation coefficient of 95.2%. This results may attribute to the arbitrary usage of the pesticide from farmers, where they believe that they need to excessively add the pesticide in order to insure the control and killing of the red palm weevil or making a precaution procedure. Just one farm of 38 SDF surveyed determine the PHI exactly for the pesticide used. Due to the traditional manners of harvesting the mechanical damage loss of date during the harvest stage is estimated at 9% at average with a minimum of 2% and a maximum of 20%, with a variation coefficient of 51,77%. the average loss of date during "Tanshir" –adjusting the moisture content- process is estimated at 10% with a minimum of 2% and a maximum of 20%, with a variation coefficient of 55,46%.

Key Words: GAP, GHP, Food Safety, Date Farms, Baharia Oasis.

1. INTRODUCTION

The date palm (Phoenix dactylifera L.) is an important fruit crop in Egypt, where Egypt is the first producer of date palm worldwide with approximately 1,694,813 tons in 2016 (FAO Statistics, 2017). Nevertheless Egypt Exports of date palm are not exceeded 36,4 thousand tons only in 2015, this may be due to some quality issues that needed to be improved to satisfy extended market segments otherwise Morocco, Indonesia and Malaysia the top three importers of the Egyptian dates respectively, where they imported together about 92% Egyptian exported date in the same year (Egyptian Food Export council, 2016). Baharia oasis is one of the distinguished date palm area in Egypt producing "Siwi" verity, according to the Baharia Oasis Agricultural Directorate, the registered possessions of date palm are 8,500 acres, while the unregistered date palm possessions estimated at 21,250 acres. The current estimated number of date palm trees in Baharia oasis in the registered land only estimated at 595000 trees (Baharia oasis agricultural directorate, 2017). The Egyptian traditional date palm sector consists mainly of small farmers possessing less than 10,000 acres is suffering from poor agricultural and handling practices alongside the date palm value chain specifically at the pre and post-harvest stages. From the technical point of view, if more systematic efforts are directed to enhance the Good Agricultural and Handling Practices of the Egyptian Siwi variety, the Egyptian date exports will elevated significantly.

2. MATERIAL AND METHODS:

This study was carried out during the first week of October 2017 in Baharia Oasis, where 38 small farms were surveyed against the applicable requirements of the United States Department of Agriculture (USDA) Good Agricultural Practices (GAP) and Good Handling Practices (GHP) guide (USDA, 2011). A questionnaire consists of 15 main questions were designed to give information about: farms profile, registration and tractability requirements, workers' health and hygiene, chemicals usage, pesticides, fertilizers, animals/wildlife/livestock, water, Soil amendments, farm cleaning, organic fertilizers, adjacent lands, harvesting, adjusting moisture before storing "Sun Dehydration or Tanshir تنشير", storage and transportation. Direct questions were directed to farmers individually or in groups at the time of rest after 3:00 pm, in addition to farm visits to some selected farms to validate the questionnaire by auditing the GAP and GHP requirements. Surveyed SDF are located in three main villages in Baharia oasis, namely; Bawity, Mandisha and El-zabo, where numbers of Surveyed SDF were 15, 14 and 9 in those villages respectively.

3. RESULTS AND DISCUSSIONS:

The study reached some major findings, which are:

3.1. Inapplicable GAP and GHP practices in Baharia small date farms:

The study found that some GAP and GHP are totally or partially missed in Baharia small date farms, such as:

3.1.1. Registration and recall requirements:

All the surveyed SDF were not using any kind of registration either for the incoming supplements or even for the sold final product. All surveyed SDF did not use any label cards at any stage in the farm.

3.1.2. Chemicals licensing requirements for pesticide application:

None of the Surveyed SDF has a license to apply pesticides, since there is no obligation in the Egyptian law concerning this requirement.

3.1.3. Water analysis:

All the Surveyed SDF were using groundwater in irrigation either from their private wells that range in depth between 200m to 800m or from the governmental wells (picture 1). No sedimentations were applied to groundwater nor any kinds of periodic analysis were in place that supposed to be thrice a year according to the USDA.

3.1.4. Arrangements to eliminate the hazards of adjacent farms:

All Surveyed SDF explained that there were not any arrangements in place to eliminate the hazards of the adjacent farms such as: simultaneously pest control or establishing physical barriers with adjacent farms.

3.1.5. Workers health and hygiene:

All Surveyed SDF shown that they were not issuing health certificates to their workers or providing them with training on hygiene. On the other hand, although all Surveyed SDF were having a bathroom attached to a designated place for eating and smoking called "Saqifa" - a place with celling to save them away from the sun at the rest time-, the bathroom lacks the hygienic requirements. The farm owners bring potable water for drinking purposes due to there was no permanent source of potable water.

3.2. Agreed GAP and GHP in Baharia Date Small Farms:

3.2.1. Animal/wildlife/livestock:

All Surveyed SDF considered the "Za'rb" or the small palm leaf wall between farms as a manner of preventing wildlife from entering their land (picture 2). All SDF do not consider birds as a hazard to their crops, so they do not use any birds' threatening tool. 92% of SDF stated that their livestock were in their barns and they did not represent a potential hazard to their crops, while 8% and the audit results found that cattle and donkeys were allowed to enter some farms and represent a potential source of damaging the product of the young short palm trees from aside and being a potential source of microbiological and physical contamination through their manure from another side (picture 3).

3.2.2. Farm cleaning:

All Surveyed SDF emphasized that they are cleaning the farm on periodic intervals to get rid of wastes resulted from refining the palm tree heads and cutting the ground grass. Sickles and hatchets are the common cleaning tools (picture 4). In regular the farmers clean the farm twice a year; the first is in September before harvesting and the second in January or April (after cultivating the alfalfa plant). The audit results revealed that the periodic interval between cleaning is too long as the grasses are so high at the time of the study (picture 5), and this is may the most probable cause of the red weevil and other pest invasion to Baharia oasis date farms, in addition to the uncared infected palm trees in the streets (picture 6). Audit indicators emphasized that the burning process of wastes was done near or in between palm trees in some cases without assigning an isolated area away of the harvesting land, and this is a potential source of physical contamination with ashes.

3.2.3. Transportation:

All SDF are agreed that the transportation process is done by the buyer either manufacturer of trader. The transported vehicles lack the hygienic design in terms of; open sided, ambient temperature and being a potential source of cross contamination.

3.3. Critical GAP and GHP in Baharia date small farms:

3.3.1. Pesticides:

All Surveyed SDF stated that they are storing chemicals in isolated place away from the crops. The audit results reveal that the chemical storage is attached to "Saqifa" or the place of rest and taking lunch, where there is often a designated room for chemicals, or even being in the same place without physical separation. Related to the pesticides groups and brands used by Baharia small date farms, the study results as in table (1) revealed that

68.4% of Surveyed SDF were using the brand Chlorzan 48% EC (Chlopyriphos), while 10.6%, 8% using Malason 57% EC (Malathion) and Tafaban 48% EC (Chlopyriphos) respectively. According to the manufacturer of chlorzane, 48% EC Kafr El-Zayat co. for pesticides and chemicals the dose of 300 cm^3 per 100 litters (0.003% dilution) is needed for controlling the red palm weevil in date palm with a pre harvest interval (PHI) of just 15 days. (Osman, 2015) found also that the residue level of Chlorozan (Chlopyrifos) was 0.01 equal to LOQ (Limits of Quantification) which was 0.01 after two weeks from treatment. Tafaban 48% EC is recommended by the manufacturer to be used at the same dose and PHI of chlorzane 48%. Malathion, the organophosphate insecticide listed by the American Cancer Society as a probable carcinogen especially linked to prostate cancer was used under the brand name (Malason 57% EC). for pesticides and chemicals also that recommend a dose of 250 cm^3 per 100 litter (0.0025% dilution) with a PHI less than 7 days in case of date palm. The diluted solutions of all the above mentioned pesticides to be used as the manufacturer as 1 litter per acre in common.

As shown in table (1) the average dilution of pesticides used in Baharia SDF was estimated at 0.01% (the standard dilution is 0.003%) with a minimum of 0.001 and a maximum of 0.05 with a variation coefficient estimated at 138%. This may attribute to unstandardized manners of dilution, where some farmers were using 2-3 pints of pesticide per 20 litters water and others were using 0.5-1 litter of pesticide per 300-350 litters of water, another group of them were using 3- 5 cm^3 pesticide per litter of water. An experimental study (Osman, 2015) tested the efficiency of Chlorozan (Chlorpyrifos) against R.ferrugineus larvae and adults after 48hr, and declared that the least effect of Chlorozan recorded on adult female where LC50 values were 341.44, 352.03 and 499.32 ppm respectively. As respect to LC90, and declared that Chlorozan was more effective on larvae than adult male while the least effect of Chlorozan recorded on adult female where LC90 values were 1245.17, 2038.91 and 2847.80 ppm, respectively.

As in table (1) also the pesticide per acre amounts were estimated in average at 2.649 litter (the standard amounts are 1 litter per acre) with a minimum of 0.571 litter and a maximum of 8 litters, with a variation coefficient of 95.2%. At the same time the diluted pesticide amounts per palm tree estimated at 10.200 liter/tree in average with a minimum of 5.6 liter/tree with variation coefficient of 43.88%. This results may attribute to the arbitrary usage of the pesticide from farmers, where they believe that they need to excessively adding the pesticide in order to insure the controlling and killing of the red palm weevil or making a precaution procedure.

In most cases the pesticide application was done in March and April during the inoculation period by overwhelming the palm tree starting from the head, and this may done for a second time after the harvesting in November. Just one farm of 38 Surveyed SDF determine the PHI exactly for the pesticide used. As shown in table (1) the Surveyed SDF determined the actual PHI to be 74,23 days in average (the Exact time for chlorsan 48% is 15 days and less than 7 days for Malason 57%) with a minimum of 15 days and a maximum of 210 days, with a variation coefficient of 294.96%. This may indicate that nevertheless the excessive addition of pesticide per acre or per tree, the long PHI may help in reducing the pesticide traces at the end of the day.

3.3.2. Fertilizers:

52.6% of the Surveyed SDF declared that they were using potassium nitrate (KNO_3), while 39.5% of them were utilizing Urea 46% nitrogen and a few farmers were using Superphosphate (5.4%). as shown in table (2)**,** the amount used of potassium Nitrate fertilizer in Baharia SDF was estimated at 1.9 kg/tree with a minimum of 1.5 kg/tree and a maximum of 2.5 kg/ tree with a variation coefficient of 73%. On the other hand, the average amounts used of Urea 46% nitrogen fertilizer was estimated at 2.278 kg per tree with a minimum of 0.5 kg/tree and a maximum of 5 kg/ tree, with a variation coefficient of 21.49%. The chemical fertilizers and pesticides used over a long period of time have adverse toxic effects on the production potential of the land and the ultimate consumers of the products (Kumari, et.al. 2014). When water soluble nitrogen fertilizers are applied to the soil, a good portion of added nutrients does not become available to the plants, but is lost to the ground water through leaching or run off. The excess nitrate leached in to rivers or ponds encourages the growth of organisms and thus a lot of organic matter produced which on decomposition lead to bad smell, which has an adverse effect on health. (Kumari, et.al. 2014).

3.3.3. Organic fertilizers:

All Surveyed SDF stated that they are using animal manures as an organic fertilizer. As in table (2) the average PHI after applying the animal manure was estimated at 100.8 days with a minimum of 30 days and a maximum of 365 day, with a variation coefficient of 79.8%. According to USDA the animal manure should be applied 2 weeks prior to planting and a minimum of 120 days prior to harvest. This means that the application of animal manures in some Baharia SDF is a potential source of contamination of the harvest area with biological contamination, and it is needed to be adjusted. Most of Surveyed SDF declared that they are applying animal manure twice a year every six months either in October and December or in March and November.

3.3.4. Harvesting:

All surveyed SDF were manually harvesting the dates with traditional methods and tools using plastic covers or mats حصير on the ground and using the old tool "El'dahr الضهر" to elevate the palm tree to cut the palm branches العرجون by Sickles or hatchets. 63.16% of the Surveyed SDF were harvesting their date in the middle of October, while 15.79%, 10.53% and 5.26% of SDF were harvesting date at the first week of November, the first week of October and the middle of September respectively. The later time to harvest the better if the precautions were taken to receive the ripened date safely instead of fallen on the dust. Due to the traditional manners of harvesting, the mechanical damage loss of date during the harvest stage was estimated at 9% at average with a minimum of 2% and a maximum of 20%, with a variation coefficient of 51,77% as shown in table (3). It's highly recommended to use cloths bags to surround fruit bunches العراجين from the early time to prevent it from insects and dust, and to save the ripened date inside in a good shape till the time of the harvest as in picture (9)

3.3.5. Sun dehydration "Tanshir تنشير":

The improper harvesting manners used as shown before resulting in collecting non-homogenized date in terms of ripening and moisture content. So farmers have to adjust the moisture content and homogenize the repining status by exposure dates to the direct sun lights in the morning for 7-10 days or what so-called "Tanshir تنشير" (picture 7). The Surveyed SDF still using their roofs and assigned areas in front of their homes for this purpose. Mats حصير, palm tree leafs or plastic covers were used to separate the date from the grounds. In this stage the insect infections, fermentation probability are increased due to the improper conditions, in addition to the higher possibility of physical contamination and mechanical loss (picture 8). The average loss of date during "Tanshir تنشير" process is estimated at 10% with a minimum of 2% and a maximum of 20%, with a variation coefficient of 55,46%. More Enhanced methods for adjusting the date moisture are required (picture 10).

4. **Storing:**

At the end of "Sun dehydration" process the dates is sorted as shown in table (3). 42% only of the Surveyed SDF were storing date for their own account, while the majority (68%) were selling the date directly after the sun dehydration process to traders or manufacturers.

5. CONCLUSION

Registration and recall requirements, Chemicals licensing requirements for pesticide application, Water analysis, Arrangements to eliminate the hazards of adjacent farms and Workers health and hygiene are the current inapplicable GAP and GHP practices in Baharia SDF. Attention were not given to animals and livestock as a source of contamination on the crop safety. Farmers giving less importance to refining the tree head, cutting grass and cleaning the farm, and this may the most probable source of food safety hazards i.e. microbiological hazards, red weevil invasion, etc.

RECOMMENDATIONS

Egyptian and Arabian as well GAP and GHP user's guide for vegetables and fruit small farmers should be developed. More Enhanced methods for adjusting the date moisture are required. It's highly recommended to use cloths bags to surround fruit bunches العراجين from the early time to prevent it from insects and dust, and to save the ripened date inside in a good shape till the time of the harvest. Arbitrary application of pesticides and chemical fertilizers that considered a probable source of chemical hazards should be adjusted via proper extension services and by enforcing legislations and laws. Out-dated traditional tools of harvesting and adjusting the moisture in dates should be gradually substituted by more technologically advanced techniques and better practices to ensure the safety of date and reduce losses.

REFERENCES

Al Raoji, K. A. (2010). Evaluation the level of understanding of people involved in date farming regarding pesticide use in date palm cultivation in Al-Qassim region, Master thesis, Nature Resources Institute, University of Greenwich (UK).

Baharia Oasis Agricultural Directorate. (2017). Unpublished raw data.

Egyptian Food Export Council. (2016). Unpublished raw data.

Food and Agriculture Organization (FAO). FAOSTAT. Last updated December 15, 2017. Accessed February 20, 2018. http://www.fao.org/publications/about-us/en/

Kumari, K. A., et.al. (2014). Adverse Effects of Chemical Fertilizers and Pesticides on Human Health and Environment, National Seminar on Impact of Toxic Metals, Minerals and Solvents leading to Environmental Pollution-2014, Journal of Chemical and Pharmaceutical Sciences.

Osman S. H. K. (2015). Studies on Red Palm Weevil and its Control, Master thesis, Dep. of Plant Protection, Al-Azhar University (Egypt).

United States Department of Agriculture (USDA). (2011). User's Guide: Good Agricultural Practices and Good Handling Practices Audit verification Program: Fruit and Vegetables Programs. Retrieved from USDA website: http://www.canr.msu.edu/foodsystems/uploads/files/Good-practices-audit.pdf

Tables

Table 1: Pesticides dilution, amounts and PHI*

statistics	PHI (actual)	pesticide amount per/ acre	Diluted pesticide amount per/ tree	dilution
Min	15.000	0.571	5.600	0.001
Max	210.000	8.000	15.000	0.050
Average	74.231	2.649	10.200	0.010
Standard Deviation	56.505	2.522	4.476	0.014
Variation coefficient	294.958	95.205	43.885	138.009

Source: the study questionnaire.

* PHI, Pre Harvest Interval

Table 2: Amounts of chemical fertilizers and PHI of animal manure used in SDF

statistics	Potassium Nitrate (kg/tree)	Urea 46% nitrogen (kg/tree)	Animal manure (PHI), days
Min	0.500	1.500	30.000
Max	5.000	2.500	365.000
Average	2.278	1.900	100.833
Standard Deviation	1.663	0.408	80.490
Variation coefficient	73.024	21.487	79.825

Source: the study questionnaire.

Table 3: loss in the harvesting and "Tanshir تنشير"* stages

statistics	Harvesting	Sun dehydration "Tanshir"*
Min	2%	2%
Max	20%	20%
Average	9%	10%
Standard Deviation	5%	5%
Variation coefficient	51.768	55.466

Source: the study questionnaire.

*** "Tanshir"** is the stage of adjusting the moisture of date before storing under sun light for a week

Pictures

Picture 1: Ground water wells and basins

Picture 2: Work animals on the orchard eating grass between the palm trees

Picture 3: "Za'rb" a tree leaf wall between two orchards

Picture 4: Traditional Sickles and hatchets used in farm cleaning and harvesting

Picture 5: High grass among palm trees

Picture 6: Uncared infected palm trees with red weevil in the streets

Picture 7: traditional adjusting of moisture under the sun "Tanshir"

Picture 8: damaged dates by pressing in the plastic boxes

Picture 9: Enhanced methods of harvesting date via cloths bags

Picture 10: Enhanced adjusting of moisture methods although unacceptable wooden frame that still used

Photos are taken by authors during the field audit

Effect of feeding date palm fruit (*Phoenix dactylifera* L.) on menstrual health in a convenient sample of females

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Abstract

BACKGROUND AND OBJECTIVES It was reported that date palm fruit (*Phoenix dactylifera* L.) affected sex hormone levels in rat model. Thus, this research aimed to study the effect of daily feeding of date palm fruit to adult females on their monthly sex hormonal concentrations in addition to studying other menstrual parameters such as menstrual cycle length and the amount of bleeding.

SUBJECTS AND METHODS A convenient sample of 37 female volunteers participated in the study. All of the participants were non-smokers, aged 20-30 years old. Each of them filled a consent form for the participation in the study.
The participants were divided into 2 groups: the experimental group who were fed daily 7 dates of "Barhi" variety for 4 consecutive menstrual cycles and the control group (the group who were not fed the fruit). The hormones measured were: luteinizing hormone, follicle stimulating hormone, 17-β-estradiol, progesterone, and prolactin. Hormone concentration was measured by enzyme linked immunosorbent assay (ELISA) technique. Other menstrual parameters such as menstrual cycle length and the amount of bleeding were also estimated using a mobile phone application that was uploaded on the cell phones of the participants.

RESULTS Feeding date palm fruit affected all menstrual hormone concentrations significantly ($p<0.05$) compared to the control group. The pattern of change in the hormone concentration didn't differ according to the month of feeding ($p>0.05$).
CONCLUSIONS Eating date palm fruit could be promising in improving menstrual health and fertility.

Key Words: date palm fruit (Phoenix dactylifera L.), lutenizing hormone, follicle stimulating hormone, progesterone, prolactin, 17-β-estradiol, menstrual characteristics

INTRODUCTION

Date palm tree is considered as an essential part of farming systems in arid and semi-arid regions (Daoud et al., 2015; Parle and Khanna, 2010). Of the date palm trees, the genus Phoenix is one of the most widely cultivated palms worldwide (Elgindi et al., 2015). Date palm fruit (Phoenix dactylifera L.) has been traditionally used for the treatment of different ailments such as memory disturbances, fever, inflammation, paralysis, loss of consciousness, and neurological disorders (Akunna & Saalu, 2012). In ancient Egypt, the fruit was used as an important ingredient in various aphrodisiac (Elgindi et al., 2015) and tonic confections. Additionally, the regular consumption of P. dactylifera palm pollen and the male flowers were reported to enhance fertility (Bahmanpour et al., 2013; Elgindi et al., 2015; Parle and Khanna, 2010). Tackholm and Drar (1973) claimed that "The pollens of a male date palm mixed with water is a charm against childlessness". Its gum is useful in the treatment of diseases of genitourinary system (Parle and Khanna, 2010). The date fruit is listed in folk remedies for the treatment of condylomata, gonorrhea, longevity, piles, sterility and urogenital ailments (Selvam, 2008; Parle and Khanna, 2010).

In the Middle East, it is common to consume about 10 to 30 dates daily as part of daily food pattern (Elgasim et al., 1995). Also, date palm fruit consumption is commonly used to break Ramadan fasting.

It has been found that date palm fruit (Phoenix dactylifera L.) affected sex hormone levels in rat model (Elgasim et al., 1995; Ammar et al., 2009; Abedi et al., 2012; Al-Sayyed et al., 2014; Daoud et al., 2015). Thus, this research aimed to study the effect of daily feeding of date palm fruit (Phoenix dactylifera L.) of "Barhi" variety to adult human females on their monthly sex hormonal concentrations and other menstrual parameters.

SUBJECTS AND METHODS

This study was a feeding trial aimed to test the effect of feeding 7 date palm fruits (Phoenix dactylifera L.) of "Barhi" variety at "Tamr" maturity stage (to a convenient sample of females) on sex hormone concentrations and to detect any menstrual changes in terms of menstrual length, menses duration, and menstrual/blood losses. The study was carried out between September 2016 and January 2018. The number 7 (7 dates) was selected to follow the habit "Sunnah" of the messenger Mohammed (Peace be upon him) in eating the fruit. The variety "Barhi" was chosen because it is the most commonly grown variety of dates in Jordan and because it was shown in a previous work, done in our laboratories, that it has an effect on the hormone 17-β-estradiol (Al-Sayyed et al., 2014).

Forty females intended to participate in the research. A written announcement was signed by the researchers after acceptance of the research proposal from the scientific research ethical committee at the University of Petra/Amman/Jordan (Decision Number 20163NI). The announcement was hanged on all the bulletin boards at different faculties of University of Petra and The University of Jordan/Amman/Jordan. The study participants were menstruating females, apparently free of diseases, sexually inactive, aged 20-30 years old, non-smokers, don't have any menstrual irregularities, don't ingest medications that affect or regulate menstrual cycle, and not following any special diet.

Before the start of the study; the research idea was explained to the volunteers, the volunteers filled a form asking about some socioeconomic and menstrual characteristics. Additionally, they filled a consent form for participation in the study.

The study participants were given the choice to participate in any of the 2 study groups (i,e. date palm fruit group or control group). Before starting the study and at each follicular phase of the menstrual cycle (2nd-5thday) during the study, each participant donated a 5 ml blood sample to measure the concentration of the hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (Prog), prolactin (Prol), and 17-β-estradiol (E2). Blood samples were drawn by a female qualified laboratory technician and blood hormone concentrations were measured by enzyme immunosorbent assay (ELISA) technique at the MedLab®laboratories, Amman, Jordan.

Before starting of the research, a mobile application was developed and uploaded on the cell phones of the study participants to remind them to eat the fruit (date group participants), fill the menstrual changes forms, to donate the blood samples, and predict the date of the next menstrual cycle (DiFilippo et al., 2015). Menstrual bleeding was defined for all of the participants as the start of spotting due to vaginal bleeding that does not require sanitary protection (WHO definition) with some modification. The end of menses was defined for the participants in accord with the Islamic religion definition of menses ending.

The assessment of menstrual blood loss was performed by pictograms with blood loss equivalents (Dasharathy et al., 2012) developed and provided by the research assistant.

Date palm fruit was purchased from a local farm in Jordan "Khayrat Assahra'a". Each study participant of the date group was requested to eat 7 dates per day throughout the study period (four consecutive menstrual cycles). The participants in both groups were asked to exclude any of the following food items and their products from their intake; soybean, chocolate, sesame and sesame paste, chickpea dip, flaxseed, walnuts, seed bread, cinnamon and licorice. The participants of the control group were exposed to the same conditions of the trial except for eating the dates. Both group participants went through a "washout" period; this was the first month of the trial; in which they didn't eat the dates, nor the prohibited foods. All data were coded and handled in a blind manner. Statistical analysis for the data was performed by the software package for social sciences (SPSS, version 23). To detect the statistical significant differences between the study groups, data of initial (baseline) characteristics of the study participants were analyzed by Hotelling's T-test. To detect the effect of feeding the fruit throughout the study period, monthly data were analyzed by mixed (effect of group and time) analysis of variance (ANOVA) for repeated measures design analysis (Laerd statistics, 2018). Significant differences were considered at $P<0.05$. Data of the baseline characteristics of the study participants are expressed in the tables 1 and 2 as mean ± standard deviation. Data of the hormone concentration and other menstrual characteristics throughout the feeding trial are expressed in the tables 3, 4, and 5 as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Thirty seven menstruating single/not sexually active females completed the research; 16 in the control group and 21 in the date palm group. Table 1 shows the socioeconomic characteristics of the study participants. This table shows that both groups were not statistically different ($P>0.05$) from each other. All of the study participants were menstruating females aged about 21 years old, students at the University of Petra, The University of Jordan, and Al-Ahileyya Amman University/Amman/Jordan. The average size of the family in both groups was 6 members and the average of their order in the family was 3. Also, their income was higher 1000 Jordan dinar (JOD).

Table 2 shows some menstrual characteristics of the study participants. Similar to the socioeconomic characteristics of the study participants, there were no statistically ($P>0.05$) significant differences between the two groups. The study participants menstruated for the first time at the age of about 13 years old, menstruate every ~ 25-26 days for about 6 days. The statistically insignificant ($P>0.05$) differences between the study groups in the initial characteristics of the study participants indicates that our sample was acceptable despite the fact that it was convenient.

The study participants had normal age of menarche, menstrual length, and menses duration. The sociocultural characteristics are thought to affect menstrual cycle as well as the menarche age (the age of first menses) which is usually between 12 and 14 years and it seems that our study participants belong to sound socioeconomic families as their menstrual length, menses duration, and age of menarche were normal. Additionally, the study participants were selected to not ingest any medicine that affects neither menses nor menstrual hormone concentrations.

Before the initiation of the study, participants were asked to exclude all the following traditionally consumed foods and their products from their intake: soybean, chocolate, sesame and sesame paste, chickpea dip, flaxseed, walnuts, seed bread, cinnamon, and licorice as these foods contain estrogen-like substances in amounts much higher than those in date palm fruit (Thompson et al., 2006).

Table 3 shows that feeding the fruit reduced significantly ($P<0.05$) the concentration of the hormones: LH (6.679±0.914 IU/L compared to 7.132±1.081 IU/L in the control group) ($P<0.001$**), Prog (0.804±0.449 nmol/L compared to1.466±0.591 nmol/L in the control group) ($P<0.008$**), and Prol (272.346±34.983 mIU/L compared to 416.520±43.632 mIU/L in the control group) ($P<0.001$**). On the contrary, feeding the fruit increased significantly ($P<0.05$) the concentration of the hormones FSH (6.573±0.587 IU/L compared to 5.885±0.695 IU/L in the control group) ($P<0.001$**) and 17-β-estradiol (50.353±3.566 pg/ml compared to 42.102±4.220 pg/ml in the control group) ($P<0.001$**). Additionally, there was no effect of the feeding duration on the hormone concentrations (data are not shown).

Menstrual cycle is defined as the monthly cyclic change pattern in terms of the secretion of reproductive hormones and the related changes in the ovaries and uterus that lasts about 25-30 days in females at the reproductive age (Mitchell, 2013).The gonadotropin-releasing hormone (GnRh) secretion results in FSH and LH secretion. The secretion of FSH stimulates the synthesis and secretion of E2 and matures the follicle and oocyte. When the follicle reaches maturity, a surge of LH and FSH triggers ovulation. The residual follicle serves as the functional corpus luteum under the primary control of LH. The LH increases the synthesis of Prog hormone. The hormones Prog and E2 promote the thickening, vascularization, and the secretory ability of uterine wall for the purpose of the implantation of the fertilized oocyte. If fertilization doesn't occur, the corpus luteum degenerates due to the reduced LH supply, and progesterone and E2 concentrations fall sharply. The hormonal stimuli for uterine wall thickening is lost and menstruation occurs (Schmidt and Litwack, 2006) usually for 2-8 days (Reed et al., 2015).

Feeding the fruit decreased significantly ($P<0.001$**) the concentration of LH compared to the non-feeding (Table 3); but still the values of the hormone were within the normal range for both of the study groups (2.4-12.6 IU/L). The literature regarding this issue is conflicting. Date palm pollen was found to reduce LH concentration after short term treatment (14 days) and increased the concentration after a longer term treatment of animals (Hammad et al., 2013). In another study, the treatment with date palm pollen extract increased the hormone concentration (Jiheel and Arrak, 2013).

Theoretically, the reduction in LH concentration would suppress Prog secretion, and degenerate the corpus luteum. These effects would be interesting to be studied in the context of fertility.

Since polycystic ovarian syndrome (PCOS) is related to increased LH:FSH ratio. Our results related to the LH and FSH can be promising in helping the ovulatory failure in PCOS patients and may result in regular menstrual cycles.

Feeding the fruit increased significantly ($P<0.001$**) FSH compared to non-feeding (in the control group) (Table 3). Both groups exhibited normal values of the hormone FSH (Table 3). Quiet similar to the findings of the current research, feeding date palm pollen extract increased significantly ($P<0.05$) the number of mice follicles without affecting the serum density of FSH. The results of the current research are probably in agreement with the results of Jashnu et al. (2016) who fed date palm fruit extract to female rats with induced polycystic ovarian syndrome (PCOS) and found a significant increase in the level of FSH (Jashni et al., 2016). Also, Hammad et al., (2012) found a significant ($P<0.05$) increase in FSH in rats after treatment of rats with date palm pollen extract. Flavonoids, alkaloids (Moshfegh et al., 2015), saponins, and steroidal compounds (Jashni et al., 2016) of the date palm fruit were reported to increase FSH. Additionally, the linoleic acid that is present in dates probably contributed to the oogenesis pathways (Modaresi and Pooe-Naji, 2012). Interestingly, El-Ridi (1960) extracted 100 IU FSH/10 g date palm pollen.

The FSH hormone promotes folliculogenesis (Jashni et al., 2016) and ovulation (Schmidt and Litwack, 2006). Thus, it is probable that feeding the fruit promotes ovulation. However, further studies related to this issue are recommended.

Feeding the fruit in the current research reduced significantly ($P<0.001^{**}$) Prog. concentration (Table 3). Nonetheless, both groups exhibited normal values for this hormone. On the contrary to our results, Housseini et al. (2014) found a significant increase in serum Prog. in rats after treatment with date palm pollen extract. Nonetheless, the reduction of the Prog. concentration due to the feeding is expected since the feeding reduced LH concentration (Schmidt and Litwack, 2006).

Prolactin (Prol) is a hormone that stimulates mammary cell differentiation and milk production (Schmidt and Litwack, 2006). Date palm fruit has been reported to be lactogenic (Elgasim et al., 1995) in pregnant and lactating mothers. In the current research, feeding the fruit reduced Prol. significantly ($P<0.001^{**}$) compared to non-feeding (Table 3). Yet both groups exhibited normal values for Prol. It is possible that the effect of feeding the fruit has different effects on Prol when fed to not-sexually active females and those who are pregnant or lactating. In non-sexually active females, increased Prol. concentration suppresses FSH and ovulation. Thus, it is probably advantageous to feed date palm fruit to females who suffer from suppressed ovulation due to increased Prol. concentration.

Feeding the fruit increased significantly the concentration of the hormone E2 compared to non-feeding. Quite similar results to those of the current research, feeding date palm fruit and date palm products or their extracts increased serum E2 in female animal models (Ammar et al., 2009; Al-Sayyed et al., 2014; Hosseini et al., 2014; Daoud et al., 2015; Moshfegh et al., 2015). Additionally, the increases of FSH might have contributed to the increase of E2.

Date palm fruit (Phoenix dactylifera L.) contains many functional compounds such as flavonoids (Hamad et al., 2015) e.g. isoquercetines (Ammar et al., 2009), lignans (Yasin et al., 2015), estrone (Wahlqvist and Dalais, 2001), estrone-like compounds, sterols and steroidal saponin glycoside (Moshfegh et al., 2015). Other date products such as pollens contain rutin (Abbas and Ateyah, 2011), amino acids, fatty acids such as linoleic acid (Fayadh and Al-Shwiman, 1989; Sulieman, 2012), saponins, and sterols (Abdi et al., 2017). These compounds are thought to promote gonadotropic actions (El-Moughy et al., 1991; Ali et al., 2013; Rahmani et al., 2014). Additionally, lignans are phenolic compounds that are found in date palm fruit and are thought to be a class of endogenous mammalian hormones (Stitch et al., 1980). The most common lignans are secoisolariciresinol and matairesinol; these two compounds can be converted by gut bacteria into enterodiol and enterolactone respectively (Setchell and Adlercreutz, 1988).

Recently, enterolactone precursors were identified in date palm fruit (Phoenix dactylifera L.) i.e. lariciresinol, pinoresinol and syringaresinol (Heinonen et al., 2001; Abbas and Ateyah, 2011; Al-Alawi et al., 2017).

The linoleic acid presence in date palm fruit might have contributed to the elevation of the effect of estradiol due to the feeding of dates because linoleic acid can be converted to estrogen (Su et al., 1999). Additionally, the tannins and flavonoids (isoflavones) were found to contribute to the estrogenic activity of date seeds (Ammar et al., 2009).

The hormone E2 promotes thickening, vascularization, and the secretory ability of the uterine wall for the purpose of the implantation of the fertilized ovum (Schmidt and Litwack, 2006). Thus, feeding the fruit probably contributes these effects and further future studies might elaborate the possible related mechanisms.

Date palm fruit contains vitamin E, carotenoids, and selenium that work as antioxidants. Several studies showed the antioxidant properties of date palm fruit at the in vivo (Vayalil, 2002; Abu–El–Soaud et al., 2004; Saafi et al., 2011) and in vitro levels

(Khanavi et al., 2010; Qusti et al., 2010). Thus, it is probable that the fruit enhanced the oxidative status of the ovarian tissue and balanced these hormones (Jashni et al., 2016).

Table 4 shows the effect of feeding the fruit on some menstrual characteristics. Feeding the fruit reduced significantly ($P<0.05$) total bleeding volume (42.071±20.304 ml compared to 70.083±21.931 ml in the control group) (P=0.003**), loss of blood in the maximum blood loss day (19.339±6.925 ml compared to 26.250±7.480 ml in the control group) (P=0.001**), menstrual secretions (10.659±3.123 ml compared to 23.312±5.179 ml in the control group) (P<0.001**), and menstrual cycle length (28.969±1.333 days compared to 31.233±1.377 days) (P<0.001**). Feeding the fruit didn't affect significantly ($P>0.05$) the menses duration (6.750±0.270 days compared to 6.617±0.287days) (P=0.051). Again, there was no effect of the feeding duration on all of these menstrual characteristics (data are not shown) except for menstrual length (Table 5). Feeding duration affected significantly (P<0.001**) the menstrual length. Also, the duration of the experimental conditions affected significantly (P<0.001**) the menstrual length of the control group.

Feeding the fruit reduced significantly the menstrual blood loss (P=0.003**) (in the menses and at the maximum day of blood loss) and menstrual daily secretions compared to non-feeding (control group) (Table 4). Heavy menstrual blood loss is a problem which necessitates medical treatment (Maybin and Critchley, 2016). The normal blood loss during the menstrual phase of menstrual cycle is <80 ml (Reed et al., 2016). Heavy loss increases the risk of iron deficiency anemia (Peuranpää et al., 2014). Both study groups had normal bleeding volume. The consumption of date palm fruit was reported to decrease post-delivery bleeding volume (Al-Farsi and Lee, 2012). Results of the current research show that the effect of feeding date palm fruit on menstrual bleeding in not-sexually active females are in agreement with those of women post-delivery. The astringent tannins, linoleic acid, oleic acid, and stearic acid (Kadem et al., 2007; Hernandes et al., 2010) of date fruits probably contribute to such an effect. Thus, feeding the fruit might be used as a complementary remedy to help in the problems of heavy menstruation.

Moreover, feeding the fruit reduced significantly ($P<0.05$) the menstrual cycle length compared to non-feeding (control group, Table 5) although the effect is not great and both groups had normal menstrual cycle length (i.e. 25-30 days, Reed et al., 2016). The feeding duration seems to have an effect on menstrual cycle length. As well, the duration of experimental conditions (probably the prohibited foods) affected the length of menstrual cycle length (Table 5).

The sample size, convenience, and the season of the research implementation are thought to be limitations of the study. Nonetheless, this is probably the first feeding trial that tested the effect of feeding date palm fruit (Phoenix dactylifera L.) of "Barhi" variety on menstrual hormones and menstrual characteristics. It can be concluded that feeding affected significantly menstrual hormone concentrations and most of the studied menstrual characteristics. Thus, date palm fruit could be tested probably in the future to ameliorate health problems that are related to menstrual cycle as it is effective, accessible, and inexpensive.

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Abbas, F. and Ateya, A. 2011. Estradiol, esteriol, estrone and novel flavonoids from date palm pollen. Australian Journal of Basic and Applied Sciences. 5: 606-614.

Abdi, F., Roozbeh, N. and Mortazavian, A. 2017. Effects of date palm pollen on fertility: research proposal for a systematic review. BMC Research Notes. 10: 363-367.

Abedi, A., Parviz, M., Karimian, S. and Rodsari, H. 2012. The effect of aqueous extract of phoenix dactylifera pollen grain on sexual behavior of male rats. Journal of Physiology and Pharmacology Advances. 2: 235-242.

Abo–El–Soaud, A.A. Sabor, A. El–Sherbeny, N. and Baker, E.I. 2004. Effect of date palm (Phoenix dactylifera L.) flavonoids on hyperglycemia. The Second International Conference on Date Palm. Arish, Egypt 6–8 October. p. 164–195.

Aggrawal, B. and Shishodia, S. 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology. 71: 1397–1421.

Akunna, G., Saalu, C., Ogunmodede, O., Ogunlade, B. and Bello, A. 2012. Aqueous extract of date fruit (Phoenix dactylifera) protects testis against atrazine-induced toxicity in rat. World J Life Sci. and Medical Research. 2:100-108.

Al-Alawi, R., Al-Mashiqri, J., Al-Nadabi, J., Al-Shihi, B. and Baqi, Y. 2017. Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options, Front. Plant Sci. 8: 1-12.

Al–Farsi, M. and Lee, C. 2008. Nutritional and functional properties of dates: a review. Critical Reviews of Food Science and Nutrition. 48: 877–887.

Al-Sayyed, H., Takruri, H., Shomaf, M. and Al-Saleh, A. 2014. The effect of date palm fruit (Phoenix dactylifera L.) on the hormone 17-β-estradiolin 7,12-dimethylbenz(α)anthracene-induced mammary cancer in rats. Mediterranean Journal of Nutrition and Metabolism. 7: 5–10.

Ammar, N., Abou El-Kassem, L., El-Sayed, N., Calabria, L. and Mabry, T. 2009. Flavonoid constituents and antimicrobial activity of date (Phoenix dactylifera L.) seeds growing in Egypt. Medicinal and aromatic plant science and biotechnology. 3: 1-5.

Bahmanpour, S., Kavoosi, F., Talaei, T. and Panjehshahin, M. 2013. Effects ofdate palm (Phoenix dactylifera) gemmule extract on morphometric parameters of reproductive tissues, hormones and sperm quality in rat. Anatomical Sciences. 10: 144-150.

Dasharathy, S., Mumford, S., Pollack, A., Perkins, N., Mattison, D., Wactawski-Wende, J. and Schisterman, E. 2011. Menstrual bleeding patterns among regularly menstruating women. American Journal of Epidemiology. 175: 536-545.

DiFilippo, K., Huang, W. and Andrade, J. 2015. The use of mobile apps to improve nutrition outcomes: a systematic literature review. Journal of Telemedicine and Telecare. 21: 243-253.

Elgasim, E. A., Al-Yousef, Y. A. and Humeida, A. M. 1995. Possible hormonal activity of date pits and flesh fed to meat animals. Food Chemistry. 52: 149–152.

El-Moughy, S.A., Abdel-Aziz, S.A., Al-Shanawany, M. and Omar, A. 1991. The gonadotropic activity of palmae in mature rats. Alexandria Journal of Pharmaceutical Research. 5: 156–159.

El-Ridi, M., El Mofty, A., Khalif, K.A. and Soliman, L. 1960. Gonadotrophs hormones in pollen grains of the date palm. Naturforschg. 15 b: 45-49.

Fayadh, J. and Al-Showiman, S. 1990. Chemical composition of date palm (Phoenix dactylifera L.). J. Chem. Soc. Pak. 12: 84–103.

Hamad, I., AbdElgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., Hegab, M., Hagagy, N. and Selim, S. 2015. Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 20: 13620-13641.

Hammed, M., Arrak, K., Al-Kafaji, N. and Hasan, A. 2012. Effect of date palm pollen suspension on ovarian function and fertility in adult female rats exposed to lead acetate. Diyala Journal of Medicine. 3: 90-96.

Heinonen, S., Nurmi, T. and Liukkonen, K. 2001. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. J Agric Food Chem. 49: 3178-3186.

Hernandes, L., da Silva Pereira, L. M., Palazzo, F. and de Mello, J. C. P. 2010. Wound-healing evaluation of ointment from stryphnodendron adstringens in rat skin. Brazilian Journal of Pharmaceutical Sciences. 46: 431–436.

Hosseini, S., Mehrabani D. and Razavi F. 2014. Effect of palm pollen extract on sexual hormone levels and follicle numbers in adult female BALB/c mice. Quarterly of the Horizon of Medical Sciences. 20:139-143.

Iftikhar, S., Bashir, A., Anwar, M., Mastoi, S. and Shahzad, M. 2011. Effect of date palm pollen (DPP) on serum testosterone levels in prepubertal Albino rats. PJMHS. 5: 639-643.

Jashni, B., Jahromi, H. and Bagheri, Z. 2016. The effect of palm pollen extract on polycystic ovary syndrome (POS) in rats. International Journal of Medical Research & Health Sciences. 5:317-321.

Jashni, H. K., Jahromi, H. K. and Bagheri, Z. 2016. The effect of palm pollen extract on polycystic ovary syndrome (PCOS) in rats. International Journal of Medical Research & Health Sciences. 5: 317–321.

Jiheel, M. and Arrak, J. 2015. Effect of different doses of ethanolic extract of date palm pollen grains on serum gonadotropin and total glutathione in mature female rats. Kufa Journal for Veterinary Medical Sciences. 6: 109-116.

Kadem, N., Sharaphy, A., Latifnejad, R., Hammoud, N. and Ibrahimzadeh, S. 2007. Comparing the efficacy of dates and oxytocin in the management of postpartum hemorrhage. Shiraz E- Medical Journal. 8: 64-71.

Khanavi, M., Saghari, Z., Mohammadirad, A., Khademi, A., Hadjiakhoondi, A. and Abdollahi, M. 2009. Comparison of antioxidant activity and total phenols of some date varieties. DARU. 17: 104–108.

Laerd Statistics. 2018. Statistical tutorials and software guides. https://statistics.laerd.com/

Maybin, J. A. and Critchley, H. O. 2016. Medical management of heavy menstrual bleeding. Women's Health. 121: 27–34.

Mitchell, M. 2013. Nutrition across the Life Span. W.B. Saunders Company. USA.

Modaresi M. and Poor-Naji, N. The effect of black seed (Nigella sativa) hydro-alcoholic extract on breeding factors in female mice. 2012. Journal of Shahrekord University of Medical Sciences. 13:63-70.

Moshfegh, F., Baharara, J., Namvar, F., Zafar-Balanezhad, S., Amini, E. and Jafarzadeh, L. 2016. Effects of date palm pollen on fertility and development of reproductive system in female Balb/C mice. Journal of HerbMed Pharmacology. 5: 23-28.

Parle, M. and Khanna, D. 2010. Phytopharmacology of Khajur (Phoenix dactylifera L .). Annals of Pharmacy and Pharmaceutical Sciences. 1: 109–115.

Peuranp, P., Heliovaara-Peippo, S., Fraser, I., Paavonen, J. and Ritva Hurskainen, R. 2014. Effects of anemia and iron deficiency on quality of life in women with heavy menstrual bleeding. ACTA Obstetricia et Gynecologica. 93: 654-660.

Qusti, S., Abu–Khatwa, A. and Lahwa, M. 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the Holy Quran. European Journal of Biological Sciences. 2: 40–51.

Rahmani, A., Aly, S., Ali, H., Babiker, A., Srikar, S. and Khan, A. 2014. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. Int J Clin Exp Med. 7:483-491.

Reed, B. and Carr, R. 2015. The Normal Menstrual Cycle and the Control of Ovulation. In: Endotext. De Groot LJ, Chrousos G, Dungan K, et al. (eds). South Dartmouth (MA).

Saafi, E., Louedi, M., Elfeki, A., Zakhama, A., Najjar, M., Hammami, M. and Achour, L. 2011. Protective effect of date palm fruit extract (Phoenix dactylifera L.) on dimethoate–induced oxidative stress in rat liver, Experimental and Toxicologic Pathology. 63: 433–441.

Schmidt, T. and Litwack, G. 2006. Biochemistry of Hormones.p. 910-911. In: Devlin, T. (Ed), Textbook of Biochemistry with Clinical Correlations (6th edition).Wley-Liss, USA.

Selvam, A. 2008. Inventory of vegetable crude drug samples housed in botanical survey of India, Howrah. Pharmacognosy reviews. 2: 61-94.

Setchell, K. and Adlercreutz, H. 1988. Mammalian Lignans and Phyto-oestrogens Recent studies on their Formation, Metabolism and Biological Role in Health and Disease. p. 315–345. In: Rowland IR (ed.),Role of the Gut Flora in Toxicity and Cancer. Academic Press: San Diego.

Stitch, S.R., Toumba, J.K., Groen, M.B., Funke, C.W., Leemhuis, J., Vink, J. and Woods, G.F. 1980. Excretion, isolation and structure of new phenolic constituent of female urine. Nature. 287: 738–740.

Su, H. M., Corso, T. N., Nathanielsz, P. W. and Brenna, J. T. 1999. Linoleic acid kinetics and conversion to arachidonic acid in the pregnant and fetal baboon. Journal of Lipid Research. 40: 1304–12.

Sulieman, A. E. 2012. Comparative study on five Sudanese date (Phoenix dactyliferaL.) fruit cultivars. Food and Nutrition Sciences. 03: 1245–1251.

Tackholm, V. and Drar, M. 1973. Flora of Egypt. Vol II., Otto Koeltz Antiquariat.

Thompson, L., Boucher, B., Liu, Z., Cotterchio, M. and Kreiger, N. 2006. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutrition and Cancer. 54: 184–201.

Vayalil, P. 2002. Antioxidant and antimutagenic properties of extract of date fruit (Phoenix dactilyfera L. Arecaceae). Journal of Agricultural and Food Chemistry. 50: 610–617.

Wahlqvist, M. and Dalais, F. 2001. The tradition and science of estrogenic plants. Journal of herbs, spices & medicinal plants. 8: 183-192.

Yasin, B. R., El-Fawal, H. A. N. and Mousa, S. A. 2015. Date (Phoenix dactylifera L.) polyphenolics and other bioactive compounds: a traditional Islamic remedy's potential in prevention of cell damage, cancer therapeutics and beyond. International Journal of Molecular Sciences. 16: 30075–30090.

Tables

Table 1: Socioeconomic characteristics of the study participant

Characteristics/Group	Date palm group (n=21)	Control group (n=16)	Probability (P)
Age (years)	21.409±0.266	21.111±0.294	>0.05
Educational level (class)	Bachelor (students of 3.68±0.716 class)	Bachelor (students of 3.61±0.698 class)	>0.05
Number of family members¬¬	6.136±0.350	6.00±0.387	>0.05
Order within the family	3.000±0.481	3.278±0.532	>0.05
Family income (JOD)1	1215.909±214.197	1686.111±236.104	>0.05

1 1 US dollar=0.71 JOD (Jordanian Dinar)

Table 2: Some menstrual characteristics of the study participants before starting of the study

Characteristics/Group	Date palm group	Control group	Probability (P)
Age at menarche	13.318±0.335	12.861±0.371	>0.05
Length of menstrual cycle	26.114±1.440	24.833±1.592	>0.05
Length of menses	6.432±0.245	5.972±0.271	>0.05

Table 3: Effect of feeding date palm fruit (Phoenix dactylifera L.) on the serum hormone concentration of the study participants

	Group			
Hormone	Date palm fruit (Phoenix dactylifera L.)	Control	Probability (P)	Normal range
Luteinizing hormone (LH) (IU/L)	6.679±0.914	7.132±1.081	<0.001**	2.4-12.6 IU/L
Follicle stimulating hormone (FSH) (IU/L)	6.573±0.587	5.885±0.695	<0.001**	3.5-12.5 IU/L
Progesterone (Prog) (nmol/L)	0.804±0.449	1.466±0.591	0.008**	0.181-2.84 nmol/L
Prolactin (Prol) (mIU/L)	272.346±34.983	416.520±43.632	<0.001**	100.6-489.3 mIU/L
17-β-estradiol (E2) (pg/ml)	50.353±3.566	42.102±4.220	<0.001**	11.3-43.2 pg/ml

Table 4: Effect of feeding date palm fruit (Phoenix dactylifera L.) on the menstrual blood loss, menstrual secretions and menstrual length and duration (menstrual characteristics) of the study participants

Group Characteristic	Date palm fruit (Phoenix dactylifera L.)	Control	Probability (P)
Total menstrual blood loss (ml)	42.071±20.304	70.083±21.931	0.003**
Maximum day menstrual blood loss (ml)	19.339±6.925	26.250±7.480	0.001**
Total menstrual secretions (ml)	10.659±3.123	23.312±5.179	<0.001**
Menstrual length (days)	28.969±1.333	31.233±1.377	<0.001**
Menses duration (days)	6.750±0.270	6.617±0.287	0.051

Table 5: Effect of feeding date palm fruit throughout the feeding trial (time effect) (Phoenix dactylifera L.) on the menstrual blood loss of the study participants

Group		
Menstrual length (days)	Date palm fruit (Phoenix dactylifera L.)	Control
First month of the feeding trial	29.438±1.957	30.933±2.021
Second month of the feeding trial	31.188±2.832	30.733±2.925
Third month of the feeding trial	26.875±1.756	32.400±1.814
Fourth month of the feeding trial	28.375±1.210	30.867±1.250
Probability (P)	<0.001**	<0.001**

Valorization of Fibrillum from palm date by-products by production of transplanting pellets

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Abstract

In recent time, problems of shortage of wood, forestry regulation and higher cost of wood materials have encouraged researchers to seek alternative sources of lignocellulosic fibers. However, in Morocco a huge quantity of by products from 4,45 millions of phoenix dactilyfera palm trees is used only for domestic purposes and very few industrial applications is observed. A better understanding of mechanical and physical properties of these by products could open new opportunities for industrial application of these lignocellulosic resources. Four by-products from *Phoenix dactylifera* were used in this study, leaflets, rachis, leaf sheath and fibrillum. The chemical composition (ashes, hydrosoluble materials, cellulose, hemicelluloses, lignin, proteins and lipids) of these by products was determined after drying and grinding. The chemical characterization showed high NDF[i] values (between 65% and 91 %). The fibrillum showed the highest content of cellulose (50.6 %) and lignin (31.9%). Mechanical properties were higher for fibrillum and leaflets. These mechanical properties of fibrillum were exploited for the elaboration of green compost and fibrillum transplanting pellets using thermopressing without the addition of water or a synthetic adhesive. The pellets manufactured have a good water retention capacity and a very satisfactory percentage of germination of tomato (78%) even after a certain delay. Beyond germination the organic matter content provided by the compost pellets allows growth of the tomato's stem of the order of 3.2 cm ± 0.7. The pellets manufactured, even if they show properties slightly lower than those of peat pellets marketed, are a novelty in the field of agro materials since green compost was added before thermoporessage. It is also a new way of valorizing the extraordinary mechanical properties of date palm fibrillum.

[1] NDF :Neutral Detergent Fiber

INTRODUCTION

Since the past few decades, problems of shortage of wood, depletion of petroleum, drawbacks of non renewable fibers and new environmental regulations, have encouraged and forced board manufacturers to seek alternative sources of lignocellulosic fibers (Bogoeva-Gaceva et al., 2007 ; Halvarsson et al., 2009; Cheung et al., 2009). In such concern, an increased interest in the use of agricultural residues and by-products from agro-industries has been growing recently (Alvarez et al., 2011).

There are about 100 million date palms worldwide (Kriker et al., 2005, Al-Kaabi et al., 2005, FAO, 2006), widespread in all of the hot areas of North Africa, the Sahara, since the Atlantic to the Red Sea, as well as to the Middle East and east to the Indus (Bendahou, 2009). In addition, the date palm is cultivated on the Canary Islands, in the northern Mediterranean and in the southern part of the United States. In Morocco, there is about 4.45 million palm trees and the average production of dates represents 75,000 t / year according to Zouine (2007).

Besides, it's nutritious and savory fruits; date palm offers a large range of by-products, exploited mainly for domestic purposes by oasian people (Chehma & Longo, 2001). This perennial tree have a fibrous structure, with four types of fibers: leaf fibers in the peduncle, baste fibers in the stem, wood fibers in the trunk and surface fibers around the trunk (Kriker *et al.*, 2005). Practically, the production of these by-products fibers equaled or even became more important than the crop itself. Every year, an average of 12–15 new leaves are formed and therefore the same amount can be pruned and removed as part of the maintenance of the palm (Barreveld, 1993; Agoudjil et al., 2011). However, their use in the agroindustrial sector was developed only from the 21st century. Much research has focused on the use of these fibers for both water treatment and metal adsorption (Al-Shayeb et al., 1995, Riahi et al., 2009a, Riahi et al. al., 2009b; Khiari et al., 2010b; Belala et al., 2011; Al-Haidary et al., 2011; Ahmad et al., 2012) and secondly for the extraction of cellulose and its derivatives, hemicelluloses and other insoluble compounds (Khiari *et al.*, 2011 ; Ammar *et al.*, 2012 ; Bendahou *et al.*, 2007 ; Ahmed *et al.*, 2013 ; Chandrasekaran et Bahkali, 2013). In the field of agromaterials, the valorization of date palm by-products started in 1989 by Ahmed et al. who proposed thermal insulation roofs from date palm fronds. However, no reference is found in the literature regarding the comparison of those different fiber sources of the same tree and self-bonding materials from the date palm fibers. Recent research has shown that the date palm by-products are characterized by a high content of polysaccharides and lignin (Bendahou et al., 2007, Sbiai et al., 2010, Khiari et al., 2010a) which makes promising their valorization for industrial purposes.

Fibrillum, which is one of these byproducts, is found on the stem at the petioles. It is cleared at the time of maintenance of the palm trees. Very few studies on this by-product and it is difficult to give a precise figure on the production of date palm fibrillum in Morocco; but it could be estimated at 5 kg / palm tree / year or 22 250 t / year if one considers a palm grove of 4.45 million trees. Thus, the objectives of this paper are the characterization of four date palm lignocellulosic by-products and the evaluation of their properties in order to produce transplanting pellets using fibrillum and green compost.

The transplanting pellets are used for the propagation of all plants (flowers or vegetables) suitable for clod production. The most commercialized are those made of peat and lime to adjust the pH. There are also pellets made of coconut fiber surrounded by a thin "membrane" textile that allows them to keep their shape.

MATERIALS AND METHODS

Raw materials: Four by-products from *Phoenix dactylifera* were used in this study, leaflets, rachis, leaf sheath and fibrillum which comes from the bark surface (fig. 1). These date palm tree fibers were collected in Marrakesh province, Morocco, cut and hammer milled through a 2-3 mm diameter mesh before analysis. The moisture content of raw materials in ascending order was 6.3%, 7.9%, 8.8% and 10% for leaflets, leaf sheath, fibrillum and rachis respectively.

Morphological analysis: The morphological characteristics of the date palm by-products were determined by evaluating their tapped density using Densitap machine (ETD-20) and particle size (AFNOR ASTM E1170). In order to visualize at the same time the state of fibers after grinding and the difference between the four by-products studied, binocular microscope observations were also carried out.

Chemical composition: The chemical composition of date palm by-products was determined on dry samples**.** The ash content and the organic matter were measured after heating the raw substrates at 550 °C during 3 h. The proteins content was determined according to the French standard NF V 18-100 while the lipids extraction was performed according to the French standard NF V 03-908. The percentage of water-soluble components was calculated from the mass loss of the test sample after 1 hour in boiling water and drying. The amounts of lignin, hemicelluloses and cellulose were assessed using ADF/NDF method of Van Soest and Wine (1967, 1968). All the experiments were carried out in triplicate.

Production of compost: we used a windrow composting with household waste, green waste and lime sludge very abundant waste in the sugar industry; the procedure is registred as patent (**MA 33393 B1**).

Transplanting pellets manufacturing by thermopressage: thermopressing, also known as compression molding, is a very old technology from the thermoset resin composite industry (Geneau, 2006). This technique involves compressing the material in a mold between the two plates of a hydraulic press, heated by conduction. The use of thermopressing in the case of agromaterials is particularly suitable for protein rich materials and natural composites consisting of both vegetable and lignocellulosic fibers (Silvestre et al., 2000).

In our study, we investigated the manufacture by dry thermopressing and without any pretreatment of the transplanting pellets which swell after addition of water to give a clump with a thickness 4 times the initial thickness of the pellets. The raw materials used are:

- ✓ Compost: household waste, green waste and sugar beet lime sludge,
- ✓ Fibrillum of date palm. The use of fibrillum is based on its chemical composition, which is very similar to coconut fiber, which is well known as a good substrate for growing and widely used to make pellets (Van Dam et al., 2004; Noguera et al. al., 2000). In addition, the fibrillum behavior towards its water absorption (271% ± 23) and its swelling in thickness (150% ± 10), showed results adapted to this kind of application.

Various proportions green compost - fibrillum were tested:

- 0% compost – 100 % fibrillum
- 10% compost – 90 % fibrillum
- 20% compost – 80 % fibrillum
- 30% compost – 70 % fibrillum
- 40% compost – 60 % fibrillum

The thermopressed pellets are prepared without any pretreatment using a hydraulic press type MAPA 50 (PEI, France). The square mold used for the pellets is 50 mm x 50 mm. This press type MAPA 50 has the following technical characteristics:

- Strenght: 500 kN,
- Voltage: 3 x 400 V,
- Power: 21 KW,
- Maximum pressure: 297 bars.

The shaping of the thermopressed pellets is carried out after conditioning of the raw material at 25 °C. and 60% humidity. The thermopressing is then performed at 180 °C using a pressure of 100 kg / cm^2 for 2 min to have a density of 1.2 g / cm^3 on average and a thickness of about 4 mm.

Quality of transplanting pellets: We tested the pellets produced by studying the water soprtion and the percentage of seed germination and seedling growth. We compared the pellet made from 10% green compost and 90% fibrillum (Pf) to the peat control pellet (Pt) marketed in France (Turba, intermas). The plant species chosen for this test is tomato (Lycopersicon esculentum L.), a Campbell 33 variety which is a standard seed variety packaged by Sogemag sarl, Morocco. Tomatoes are often used in agronomic tests because they are robust, fast-growing and grow very well in the greenhouse. The percentage of germination tested under laboratory conditions of this variety is equal to 90% on average.

Experimental Procedure: the manufactured pellet was cut to have the same diameter and the same shape as the pellet marketed. The thickness and weight of these pellets were also measured. 20 ml of distilled water are added; after swelling of the pellets, six tomato seeds previously disinfected with bleach were seeded on each pellet. All the pellets were placed later in the culture chamber. The climatic conditions of this chamber are kept relatively stable at 22 ° C, 60% relative humidity and illumination of 14 hours during the day and 10 hours at night throughout the test period. Three repetitions were made for each type of pellets. A fungicide (SAAF, imported by the Korale-Morocco) is used after eight days of cultivation to avoid any contamination by fungi.

For the measurement of water sorption, transplanting pellets pieces equilibrated at 60% RH were placed in a hermetic container above a saturated KCl solution (85% RH) during 7 days. They were regularly weighted and their mass gain calculated in relation to their initial mass. Reported values are the average of three experiments.

RESULTS

Characterization of the palm by products: The chemical composition of the date palm by-products are presented in Table 1. As expected for lignocellulosic fibers the results show that these raw materials are characterized by high amount of NDF (65 % -91 %). The major compounds are cellulose and hemicelluloses for leaflets, leaf sheat and rachis, while, the fibrillum showed the highest content of cellulose (50.6 %) and lignin (31.9 %). These results are relatively close to the results obtained by Bendahou at al. (2007) and Sbiai et al. (2010), except for the lignin leaflets contents (27 %) ; thus, the results of these authors showed 33.5% -35% of cellulose and 26-28 % of hemicelluloses for leaflets and 44% of cellulose, 28% of hemicelluloses and 14% of lignin for rachis. The composition of sisal (Williams and Wool, 2000), another leaf fiber, shows that it contains more cellulose (67-78 %) and less contents of hemicelluloses (10-14.2 %) and lignin (8-11 %) compared with the date palm leaf fibers. The main difference between these four date palm by-products is related to protein content and hydrosolubles materials which are higher for Leaflets (9.7% and 24.3% respectively) compared to other by products. Furthermore, the fribrillum showed the highest content of cellulose and lignin. The ash contents is high for leaf sheath (12.3 %) and presents the same value for leaflets and rachis (9.2 %), while, it is lower for fibrillum (6.8 %) but remains at the same order of magnitude as most of the non-wood fibers like wheat straw and oat straw (Khiari et al., 2010). The granulometric repartition of the four date palm by-products hammer milled shows a significant difference between the four by products (Fig 2). However, very few proportion of particles have diameter over 1,25 mm and the major particles have diameter lower than 1,25 mm. The microscopic observations (Table 2) reveal also the undeniable difference between the by-products. This morphological difference may explain the mechanical properties of bio-based materials produced. The highest tapped density (0,316) is observed for leaflets while the Leaf sheath has the lowest (0,199) tapped density (Table 2).

Characterization of compost: The compost used (Table 3) is very rich in organic matter (45.9% DM) necessary for plant growth; however, it shows low levels of cellulose (1.9%) and protein (5.4%) in contrast to fibrillum.

Quality of transplanting pellets: The germination results (Fig. 3) show that after a marked delay, pellets produced from compost and fibrillum give a percentage of germination (78%) slightly lower than that of peat pellets (87%). This delay of germination could be reduced if we improve the porosity of the elements in our pellets and thus on the water retention.

Spiers and Fietje (2000) reported that an excess of fine particles (less than 0.1 mm) obstructs pores and decreases water retention. The results of the growth of tomato plants in the various pellets tested show that after 13 days peat pellets grow to an average of 3.2 cm ± 0.2 whereas the pellets produced have a slightly lower growth (2 cm ± 0.1). This result can be improved by adjusting the water retention. Regarding the evaluation of the water retention by the pellets, it is found that after 120 min the pellets manufactured containing 10% of compost have a water retention capacity of 247.5% against water retention of 299.5%. 9% for the marketed pellets (Fig. 4).

DISCUSSION & CONCLUSION

Chemical characterization of four date palm by-products has been assessed. The comparison showed high contents in lignin and cellulose for fibrillum and high contents in protein, and hydrosolubles materials for leaflets. The thermopressing process allowed us to produce transplanting pellets from compost and fibrillum without any addition of water or synthetic resin. These results showed the possibility of using date palm by-products. However a better understanding of mechanical properties and the chemical characterization of fibrillum could improve the quality of these transplanting pellets and open new opportunities for industrial application of this new lignocellulosic resource. In this context, more studies could be performed to further optimize the production of transplanting pellets and their application in agriculture.

REFERENCES

AFNOR ASTM E1170

Agoudjil B., Benchabane A., Boudenne A., Ibos L., Fois M., 2011. Renewable materials to 260 reduce building heat loss: Characterization of date palm wood. Energy and Building 43 (2-3), 261 491-497.

Ahmad T, Danish M, Rafatullah M, Ghazali A, Sulaiman O, Hashim R. Ibrahim MNM. The use of date palm as a potential adsorbent for wastewater treatment: a review. Environmental Science and Pollution Research 2012; 19:1464–1484.

Ahmed J, Almusallam A, Al-Hooti SN. Isolation and characterization of insoluble date (Phoenix dactylifera L.) fibers. LWT - Food Science and Technology 2013;50: 414-419.

Al-Haidary AMA, Zanganah FHH, Al-Azawi SRF, Khalili FI, Al-Dujaili AH. A study on using date palm fibers and leaf base of palm as adsorbents for Pb(II) ions from its aqueous solution. Water Air and Soil Pollution 2011; 214:73–82.

Al-Kaabi K, Al-Khanbashi A, Hammami A. Date palm fibers as polymeric matrix reinforcement: DPF/polyester composite properties. Polym Compos 2005;26: 604–613.

Al-Shayeb SM, Al-Rajhi MA, Seaward MRD. The date palm (*Phoenix dactylifera L.*) as a biomonitor of lead and other elements in arid environment. The Science of the Total Environment 1995; 168: 1-10.

Álvarez C, Rojano B, Almaza O, Rojas OJ, Gañán P. Self-bonding boards from Plantain fiber bundles after enzymatic treatment: adhesion Improvement of lignocellulosic products by enzymatic pre-treatment. Journal of Polymers and the Environment 2011;19:182–188.

Ammar H, Abid M, Abid S. Cellulose fibers obtained by organosolv process from date palm rachis (*Phoenix dactylifera L.*). IOP Conf.Series: Materials Science and Engineering 2012; 28, 012002.

Barreveld W.H, 1993. Date Palm Products, in Food and Agriculture Organization of the 271 United Nations Rome, FAO Agricultural Services. Bulletin 101.

Belala Z, Jeguirim M, Belhachemi M, Addoun F, Trouvé G. Biosorption of copper from aqueous solutions by date stones and palm-trees waste. Environmental Chemistry Letters 2011; 9: 65–9.

Bendahou A., Kaddami H., Raihane M., Habibi Y., Dufresne A., 2009. Revue Roumaine de 276 Chimie 54 (7), 571- 575.

Bogoeva-Gaceva G, Avella M, Malinconico M, Buzarovska A, Grozdanov A, Gentile G, Errico ME. Natural fiber eco-composites. Polymer Composites 2007; 28: (1) 98-107.

Chandrasekaran M, Bahkali AH. Valorization of date palm (Phoenix dactylifera) fruit processing by-products and wastes using bioprocess technology – Review. Saudi Journal of Biological Sciences 2013 ; 20, 105–120.

Chehma A., Longo H.F., 2001. Valorisation des sous-produits du palmier dattier et leur 280 utilisation en alimentation du bétail. Revue des énergies renouvelables (Unesco). Numéro 281 spécial Biomasse : Production et Valorisation, 59-64.

Cheung H.Y., Ho M.P., Lau K.T., Cardona F., Hui D., 2009. Natural fibre-reinforced 283 composites for bioengineering and environmental engineering applications. Composites, Part 284 B 40 (7), 655-663.

Evon P., Vandenbossche V., Rigal L., 2012. Manufacturing of renewable and biodegradable fiberboards from cake generated during biorefinery of sunflower whole plant in twin-screw extruder: Influence of thermo-pressing conditions. Polymer Degradation and Stability 97 (10), 1940-1947

FAO, 2006

Geneau C. Procédé d'élaboration d'agromatériau composite naturel par extrusion bi-vis et injection moulage de tourteau de tournesol. Thèse de Doctorat. Institut Polytechnique de Toulouse, France, 2006.

Halvarsson S, Edlund H, Norgren M. Manufacture of non-resin wheat straw fiberboards. Industrial Crops and Products 2009;29: 437–445.

Halvarssona S., Edlunda H., Norgrena M., 2009. Manufacture of non-resin wheat straw 290 fiberboards. Industrial Crops and Products 29 (2-3), 437-445.

Khiari R, Mhenni MF, Belgacem MN, Mauret E. Valorisation of vegetal wastes as a source of cellulose and cellulose derivatives. Journal of Polymers and the Environment 2011 ;19:80–89.

Khiari R., Dridi-Dhaouadi S., Aguir C., Mhenni M.F., 2010. Experimental evaluation of eco- 298 friendly flocculants prepared from date palm rachis. Journal of Environmental Sciences 22 299 (10), 1539-1543.

Khiari R., Dridi-Dhaouadi S., Aguir C., Mhenni M.F., 2010. Experimental evaluation of eco- 298 friendly flocculants prepared from date palm rachis. Journal of Environmental Sciences 22 299 (10), 1539-1543. 300

Khiari R., Mhenni M.F., Belgacem M.N., Mauret E., 2010. Chemical composition and 301 pulping of date palm rachis and Posidonia oceanica – a comparison with other wood and non-302 wood fibre sources, Bioresource Technology 101 (2), 775-780.

Kriker A, Debicki G, Bali A, Khenfer MM, Chabannet M. Mechanical properties of date palm fibres and concrete reinforced with date palm fibres in hot-dry climate. Cement & Concrete Composites 2005;2:554–564.

Kriker A., Debicki G., Bali A., Khenfer M.M., Chabannet M., 2005. Mechanical 304 properties of date palm fibres and concrete reinforced with date palm fibres in hot-dry 305 climate. Cement & Concrete Composites 27 (5), 554-564.

Laemsak N., Okuma M., 2000. Development of boards made from oil palm frond II: 292 properties of binderless boards from steam-exploded fibers of oil palm frond. Wood Science 293 46 (4), 322-326.

MA 33393 B1)
Noguera P, Abad M, Noguera V, Puchades R, Maquieira A. Coconut coir waste, a new and viable ecologically friendly peat substitute. Acta Horticulturea 2000; 517: 279–286.
Riahi K, Ben Mammou A, Ben Thayer B. Date-palm fibers media filters as a potential technology for tertiary domestic wastewater treatment. Journal of Hazardous Materials 2009a;161:608–613.
Riahi K, Ben Thayer B, Ben Mammoub A, Ben Ammarc A, Jaafourac MH. Biosorption characteristics of phosphates from aqueous solution onto *Phoenix dactylifera L.* date palm fibers. Journal of Hazardous Materials 2009b; 170 : 511–551
Sbiai A, Maazouz A, Fleury E, Sautereau, H, Kaddami, H. Short date palm tree fibers/polyepoxy composites prepared using RTM process: effect of tempo mediated oxidation of the fibers. Bioresource 2010; 5: 672–89.
Silvestre F, Gaset A, Rigal L, Leyris J. Method for Making Shaped Objects from a Vegetable Raw Material by Pressing. European Patent 0,987,089, 2000.
Spiers TM, Fietje G. Green waste compost as a component in soilless growing media. Compost science & utilization 2000; 8: 19–23.
Van Dam JEG, Van den Oever MJA, Teunissen W, Keijsers ERP, Peralta AG. Process for production of high density/high performance binderless boards from whole coconut husk: Part 1: lignin as intrinsic thermosetting binder resin. Industrial Crops and Products 2004;19: 207–216.
Van Soest P.J., Wine R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. 322 Determination of plant cell wall constituents. Journal of the Association of Official Analytical 323 Chemists 50 (1), 50-55. 324
Van Soest P.J., Wine RH, 1968. Determination of lignin and cellulose in acid detergent fiber 325 with permanganate. Journal of Journal of the Association of Official Analytical Chemists 51 326 (4), 780-784.
Widsten P., Kandelbauer A.,2008. Adhesion improvement of lignocellulosic products by 330 enzymatic pre-treatment. Biotechnology Advances 26 (4), 379-386.
Williams GI, Wool RP. Composites from natural fibers and soy oil resins. Journal of Applied Composite Materials 2000; 7 (5-6): 421–32.
Zouine J. Embryogenèse somatique chez Phoenix dactylifera L.: Amélioration des conditions de culture et étude de paramètres biochimiques impliqués dans la multiplication, la maturation et la germination des embryons somatiques. Thèse de Doctorat. Université Cadi Ayyad de Marrakech, Maroc, 2007.

Tables

Table 1

Chemical composition (wt%) of date palm by-products

Constituent	Raw materials			
	Fibrillum	Leaflets	Rachis	Leaf sheath
Dry matter	91.2 ± 0.2	93.7 ± 0.5	89.9 ± 0.1	92.1 ± 0.5
Ash	6.8 ± 0.2	9.2 ± 0.4	9.2 ± 0.1	12.3 ± 0.2
NDF*	90.6 ± 3.7	64.6 ± 0.8	85.2 ± 1.2	81.1 ± 1.6
Cellulose	50.6 ± 1.3	29.7 ± 1.3	39.8 ± 0.9	34.0 ± 0.7
Hemicelluloses	8.1 ± 0.3	23.3 ± 1.2	31.4 ± 3.2	28.9 ± 1.8
Lignin	31.9 ± 1.3	11.6 ± 1.3	14.0 ± 0.9	18.2 ± 0.7
Proteins	6.2 ± 0.1	9.7 ± 0.3	1.5 ± 0.1	2.0 ± 0.1
Wax	0.4 ± 0.1	6.8 ± 0.7	1.0 ± 0.2	0.5 ± 0.1
Hot water extractives	9.7 ± 1.5	24.3 ± 0.2	16.8 ± 0.3	18.7 ± 1.4

*NDF: Neutral Detergent Fiber

Table 2: Bulk density and microscopic observations (binocular magnifying glass) of hammer milled date palm by-products

Raw materials	Fibrillum	Leaflets	Rachis	Leaf sheath
Bulk density (g/cm^3)	0.241 ± 0.003	0.316 ± 0.003	0.289 ± 0.018	0.199 ± 0.006
Median diameter d_{50} (mm)	0.389	0.374	0.426	0.354
Microscopic observation (x 10)				

Table 3: Chemical composition of compost (% DM)

Elements	**Compost**
Organic Matter	45,9 ± 0,3
Ash	54,1 ± 0,3
NDF*	22,0 ± 0,4
Cellulose	1,9 ± 0,1
Hemicelluloses	17,6 ± 0,9
Lignin	2,5 ± 0,4
Protein	5,4 ± 0,1
Fatt	0,12 ± 0,05
Watersolubles	7,9 ± 2

*NDF: Neutral Detergent Fiber

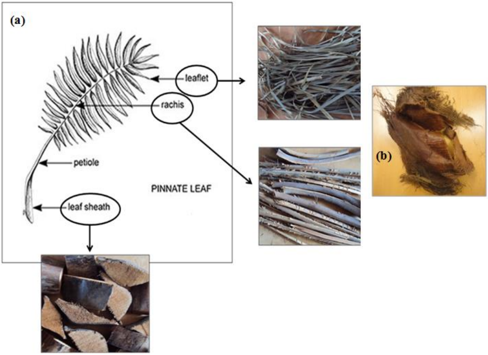

Fig. 1. Schema and photograph of the various date palm leaf by-products (a) and date palm tree surface fiber (fibrillum, b)

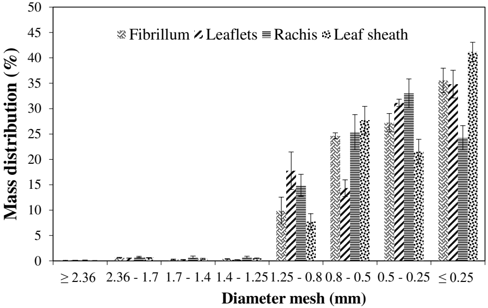

Fig. 2. Granulometric repartition of date palm by-products hammer milled through a 3 mm diameter me

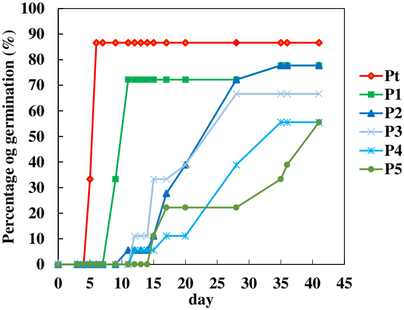

Fig. 3. percentages of germination obtained with different transplanting pellets
Pt : marketed transplanting pellets, P1 : 0% compost – 100 % fibrillum,
P2 : 10% compost – 90 % fibrillum, P3 : 20% compost – 80 % fibrillum,
P4 : 30% compost – 70 % fibrillum, and P5 : 40% compost – 60 % fibrillum

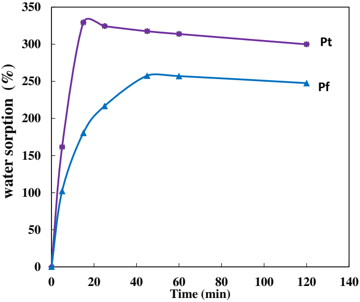

Fig. 4. Evolution of the water sorption for the marketed transplanting pellets (Pt) and the transplanting pellets produced from palm by product (Pf)

Date palm value chain development in the Arab countries: key constraints and opportunities

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Abstract

Date palm represents an especial icon in agriculture in the Near East and North Africa region. Since its origin is believed to be in the Arab region, dates have a unique historical, religious, economic, and social value. With an estimated production of nearly 6.5 million tons in 2016, dates represent a main source of income and staple food for virtually all countries in the region. The rich nutritional composition of dates also highlights its importance to food security and nutrition. Despite significant efforts, the date sub-sector has an enormous room to grow in the region, and efforts should focus on integrated national and regional strategies. The expansion of the date palm industry is dependent on many aspects which, including control of pest and diseases; enhancing the quality of date end products; reducing field and post-harvest high losses; formalizing and streamlining market systems; and, developing date value added products and byproducts. This paper argues for a more holistic, market-driven and inclusive approach to sustainably develop the date palm value chain in the Arab region and at the country level, with a focus on increasing competitiveness, access to higher value markets, and expanding growth. The paper also highlights systemic constraints and opportunities related to core functions along the value chain, the enabling environment, horizontal and vertical linkages, and supporting services. It elaborates around concerted regional strategic integrated efforts needed to address systemic bottlenecks in the date palm value chain. These efforts are analyzed through a wide value chain and food system approach and consider elements such as food security and nutrition, food safety, reduction in food loss and waste, sustainability on of natural resources, contribution to ecosystems and biodiversity, while supporting income and employment generation.

1. INTRODUCTION

Date palm is an important global industry with the yearly global production exceeding 8 million tons in 2016. Processed dates are marketed all over the world for direct consumption and further processing into high-value products. The leading global date producers are Near East and North Africa (NENA) countries, which favored by the suitable dry sub-tropical and high temperature climate, produce about 75% of the global supply (FAO, 2018). The date palm has been growing in the deserts of the NENA region for over 7,000 years and is closely associated with the life and culture of people. It is one of the oldest cultivated fruit crops and a major staple food for the region. Since its origin the date palm has played an important role in food production, foreign exchange, raw materials, and income and employment generation.

The date palm has provided humankind for ages with good sources of energy and other essential nutrients. The nomads have been historically depending on supplies of dates and dairy for their nourishment. Dates have been considered an excellent snack food as it contains reasonable amounts of vitamin A, thiamin, riboflavin, and niacin and are a good source of potassium, calcium, iron and dietary fiber (Al-Khayri, Jain et al., 2015). The growing natural food sector is promoting date consumption as a functional food, which offers antioxidant qualities. In addition, dates are effective for treatment of many health problems because of its antibacterial, antifungal, antitumor, antiulcer, antioxidant and immuno-modulatory attributes (Al-Khayri, Jain et al., 2015).

Date palms can grow below 392 meters to above 1500 meters above sea level, with a range of altitude of 1892 meters. The date palm is considered a symbol of life in the desert, because of its ability to tolerate several abiotic stresses including, high temperatures, drought and salinity, more than many other fruit crop. The date palm not only provides a concentrated nutrient dense food, it also prevents soil degradation and desertification and creates a more amenable habitat by providing shade for protection from the desert wind and heat. Crops such as legumes and bananas, as well as orange and citrus trees, depend on the palms for protection from winds. It is capable of establishing a sustainable system in subsistence agricultural areas and thus plays an important social role for a large portion of the population by helping to settle in rural areas versus migration to urban centers.

Biodiversity of date palm is a prerequisite for the proper functioning of the oasis ecosystem, which is complex and characterized by horticultural, agronomic, ecological, economic, social, and cultural dimensions. It represents the climax of rigorous management of scarce water and land resources in alliance with the date palm. The date palm is the dominant component upon which the sustainable biophysical and socioeconomic structures of the oasis are based.

Intercropping of dates with irrigated crops is a common practice in the Sahara as an effective way to withstand changing climate. Date palm is cultivated in a wide range of cropping and farming systems such as oasis, groves, home gardens, as a mono-crop and as an intercrop. There are more than 400 date varieties that are known, but the number of date varieties that have reasonable yields are probably less than 50, out of which 25-50% are of commercial value (Sawaya, 2000).

Date palm is also a good income-generating crop as well as a source of employment in the region. Practically almost every part of the date palm except perhaps its roots provide one or more services of economic or environmental value. Over the past centuries, hundreds of uses have been found for different parts of the palm in agricultural production, as well as domestic utensils and small industries. While the fruits are eaten as dates; mats, beds and chairs can be made from palms; and the leaves used to make baskets and to feed livestock. Date palm is a labor-intensive crop that requires pruning, pollinating, harvesting and storing, processing (some prized as delicacies, others used as the basis for products ranging from vinegar to syrup, medicine to animal feed) and marketing of the dates. A significant number of families depend on the crop for the bulk of their income, since a large number of groves in the region belong to small and medium-size holders and provide seasonal employment, especially for women.

The international demand for dates and its byproducts is constantly expanding which increasingly becomes a pull factor to the development of the date palm industry in the region. The major Arab exporting countries export about 340 thousand tons of date fruits to countries out of the region, and another 171 thousand tons to countries in the region. India is the main importer of dates with a share of about 40% of global imports. While European Union (EU) countries account just for 10% of global imports in terms of volume, they account for some

30% in value (Liu, 2003). This reflects the fact that EU import prices for dates are much higher than the world's average. There is room for increased imports of dates in many countries provided that high standards of quality, packaging and traceability are met.

The commercial development of date industry is imperative to allow countries to profit from the continued expansion of global demand for date products. The improvement of the status of date palm cultivation in the date producing countries of the region and the enhancement of the quality of date products is a critical need that cannot be overemphasized. The region showcases attractive experiences from exporting countries like the Egypt, Kingdom of Saudi Arabia (KSA), Tunisia and the United Arab Emirates (UAE), which have been aggressively working towards increasing their market shares as established markets expand and new ones arise. Nevertheless, there are still key systemic production, post-production, and marketing issues region wide and at the country level that greatly affect the date palm value chain in the region, with strong negative consequences on the countries and region's ability to fully benefit from market opportunities.

This paper argues that the efforts to foster sustainable growth of the date palm value chain in the region should focus on integrated national and regional strategies. The expansion of the date palm value chain is dependent on controlling the spread of Red Palm Weevil (RPW) and other important pests and diseases, which continues to have negative impacts on productivity and viability of traditional and commercial plantations; enhancing the quality of date end products; reducing field and post-harvest high losses; formalizing and streamlining market systems; and, developing date value added products and byproducts. Based on evidence of recent developments, this paper advocates for a more holistic, market-driven and inclusive approach to sustainably develop the date palm value chain in the Arab region and at the country level, with a focus on increasing competitiveness, access to higher value markets, and expanding growth.

This is a preliminary product of a collaboration that the Arab Organization for Agricultural Development (AOAD) and the United Nations Food and Agricultural Organization (FAO) are engaged to jointly promote country-driven and regionally integrated actions intended to address key bottlenecks and accelerate growth in the date palm value chain in the Arab region. This collaboration is developing, as a first step, a regional strategic framework for the development of the dates palm value chain in the region. The framework will serve as an umbrella for concerted interventions at country and regional levels, and is being developed in the context of a wide food system approach, considering the potential for food security and nutrition, sustainability of natural resources (soil and water), ecosystems and biodiversity, potential for income generation and employment, the importance of reducing dates losses, and aspects of food and safety and quality. A key input for the development of the framework is the value chain study that AOAD and FAO are conducting in the region with inputs from region experts and country studies.

2. The date palm value chain in the region

Date palm (Phoenix dactylifera L.) is the main fruit crop in arid and semiarid regions, particularly in western Asia and North Africa. The palm tree is well adapted to desert environments that are characterized by extreme temperatures and water shortage, both in quality and quantity. Beyond the arid climates, date palm can also be grown in many other countries for food or as ornamental. The majority of date palm-growing areas are located in developing or underdeveloped countries where dates are considered the primary food crop, thus playing a major role in food security and the nutritional status of these communities.

The world population of date palm is estimated at 100 million trees distributed over an area of about 600,000 hectares (ha), of which 422,000 ha or 70% are in the Arab region. This area represents 5% of planted land in Arab countries with more than 62 million date palms that produce 4.5 million metric tons or 67 % of annual global production (Liu, 2003). The average productivity of date palms varies from 3 to 38 ton/hectare, according to cultivar and tree age, environmental conditions, cultivations patterns, and care in each of the date-producing countries.

The world date palm cultivation is concentrated mostly in the NENA, favored by suitable dry sub-tropical and high temperature climate. Date palm thrives in areas characterized by hot, low humidity, particularly during fruit development. Moisture adversely affects the quality of fruit, as high humidity leads to fruit cracking and checking. Date palm can be planted in a wide range of soils with varying amounts of organic and mineral nutrients. Date palm is known to tolerate salinity more than any other cultivated fruit crop. Many parts where date palm is grown still follow the traditional mixed planting of dates of various ages at irregular spacing.

Date palm production and trade has witnessed a continuous increase at the global level. According to (FAO, 2016), as of 2016 about 74% of the world production of dates is in the Arab countries; half of these is in the Arabian Gulf area (including Iraq and Yemen) and the other half in North Africa countries, including Sudan. The area under date palm cultivation has expanded in almost every single country in the region for the last few years, with a remarkable expansion been experienced in UAE and Iraq which more than doubled their plantations from 2012-2016, and Egypt that increased by 25% its areas in the same period to become largest producer of dates in the region (see Table 1). The region expanded its land under date palm cultivation from about ha 775,000 in 2012 to slightly more than one million in 2016. The only case of significant contraction of the date palm area is Mauritania which in the period of 2012-2016 reduced its date area from about 8,500 to nearly 4,200 hectares, roughly 50% decrease.

The expansion in the area under production is also coupled with an expansion in the production of dates in the Arab region. The production has grown at an average rate of 4% from 2012-2016, which resulted from a 5% growth of dates cultivated areas and a 1% yield decline during the period 2012-2016. The world's largest producer over the same period (2012-2016) was Egypt with an average of 1.5 million tons of dates, followed by KSA with a production of about 0.95 million tons, Algeria (0.92 million tons), UAE (425,000 tons), and Sudan (437,000 tons). Other significant producing countries are Iraq, Oman, and Tunisia, Libya, and Morocco (see Figure 1).

Table 1. Areas of production of date palm in the Arab region and other date producing countries
Source: Authors based on FAOSTAT 2018

The differences in terms of yield between countries in the region is noticeable, and in some cases have reduced substantially in the last few years. As of 2016, Egypt leads the yields in the region with about 35 tons/ha, followed by Kuwait 32 tons/ha, and Oman 14 tons/ha. These average yields are very different from the yields of countries like Iraq, Morocco, Bahrein, Yemen and Tunisia whose yields are notably under 5 tons/ha (see Figure 1).

Figure 1. Levels of date production and productivity in Arab countries
Source: Authors based on FAOSTAT 2018

2.1. Date palm value chain main market channels

The date palm value chain in Arab region involve a variety of actors which are performing in different parts of the value chain from input supply to production, collection, processing, wholesaling and retailing, and export. It also involves actors that are providing supporting services to different functions of the value chain, including extension and training services, packaging, and financial services (see Figure 2).

The two main types of dates producers, namely, traditional small-scale dates orchards and the modern plantations, engage in different types of horizontal and especially vertical linkages, which starts with the providers of supporting services (extension and training services, packaging, financial services, etc.) and the inputs suppliers. Traditional small-scale date orchards channel their fruits to collectors and brokers, retail traders, wholesaler traders, dates processors, to rural and urban domestic markets as well as to modern dates producers which through this increase their volumes in order to meet the demands of their buyers. The modern dates' plantations in turn supply mainly the wholesale traders, processors and exporters, which normally find large volumes as attractive way to reduce transaction costs.
The dates collectors assume the brokerage role and facilitate the flow the dates to the retail traders, wholesale traders, processors and the domestic market outlets. Dates wholesale traders make their stock from the producers, collectors, retailers and link up with processors, other second-tier wholesale traders and exporters. Processors sell their produce to the retail traders, second-tier wholesale traders for export, and the domestic market. The end market for the dates' value chain involves both the rural and urban domestic market and the export market of the main importing countries.

There is important channel distinction between dried dates and fresh dates, which are not distributed and marketed in the same way. The channel for the distribution of dried fruit follows a commercial pattern adapted to the nature of its products. On the other hand, there is a distribution channel for fresh fruit presenting a completely different situation, with very specific buying habits, and above all completely different logistical constraints. In this context, dates will be bought, for example, by fruit and vegetable wholesalers each week on the national markets of major conurbations. The packaging of dried fruit and fresh fruit also differs. Whereas dried fruit can be hidden away in closed packaging, such as packs and boxes of different kinds to target both the local and export markets, fresh fruit needs to be seen and are normally sold loose or in transparent packaging exclusively in the local market.

Figure 2. Date palm value chain map in the Arab region
Source: Authors

2.2. Key value chain actors and their functions

- Inputs suppliers: these mainly supply seedlings, pesticides, machinery, and equipment to date producers. These are normally private sector players, which can be divided into two main categories: a) domestic producers of inputs, and b) foreign companies importing inputs into the region. Nonetheless, in some countries the government is also involved in the provision of some inputs like seedlings and chemicals to date producers, especially scale-scale who are often ill-equipped to invest in inputs.
- Producers: the majority of dates' producers in the region are small-scale, however modern plantations (often more extensive) are increasingly playing a preponderant role in the dates value chain in the region. Traditional small-scale date's orchards are often resourced constrained with no access to cool storage and packing facilities, while modern plantations are big producers with much more capacity to invest and manage

their plantation, often with access to cool storage and packing facilities. These producers face key challenges which greatly affect their capacity to increase productivity, reduce production costs and access high value markets, including: limited adoption of modern cultivation practices and technologies; lack of management skills and professionalization; high soil and water salinity; endemic pests and diseases (especially RPW, Dubas Bug and Bayoud disease); poor quality of products; and limited economies of scale. Some of these problems do affect modern plantations, however there others such as the RPW which affect producers across the spectrum.

- Dates collectors/brokers: these are intermediaries who play an important role in linking the producers with the retail traders, wholesale traders, processors, and directly to outlets in the domestic market. The collectors often have limited capacity to invest and have limited access infrastructure. While they provide small-scale farmers access to marketing options lack of alternatives leads to lower prices for farmers.
- Dates wholesale traders: wholesalers work as intermediaries between farmers, collectors, traders and retailers. The second-tier wholesale traders buy from processors, and deal with the end markets, primarily the export one. Wholesalers often sell on an auction basis and the supplier (farmer or collectors) and buyer (retailers) pay a fee to the wholesaler. Traders usually buy from producers on a short-sell basis (i.e. they book the crop around the start of the season and pay cash to the farmer upfront). Over the past years, traders have developed a relationship of trust with producers. With no large processors for the traders to sell to, traders usually sell to second-tier wholesalers or retailers. As the most of dates are usually sold as table dates and not processed, traders face difficulty to maximize profitability.
- Dates processors: processing plants are mostly small and medium enterprises (SME) and they purchase dates inputs directly from the producers. Tangible quantities of dates are processed as home-based by women. Dates processors suffer from problems such as poor quality of raw materials (dates), difficulty to minimize costs, and limited product processing opportunities.
- Retailers, distributors, dealers: sales of fresh dates are usually carried out through intermediaries' distributors and dealers in the local and external markets. Retailers include those selling at local market places, vegetable stores, and supermarkets in rural and urban domains. Their main constraints relate to the scale of business and the difficulty to maintain a reasonable flow of product in their shelves.
- End-market: despite of the local market which is very important for the commercialization of dates, the Arab producing countries reach many export markets directly through large producers, second-tier wholesale traders, and wholesale trade companies who are connected with the international market mainly in India, Europe, USA, and even some Arab Countries. The export market is more exigent in terms of quality and value addition but is also much more generous in terms of prices which in the local markets remarkably low. Improving food safety along the date palm value chain and meeting the stringent safety and quality standards for date products has been a key challenge which needs to be addressed to result in increased income for exporting countries.
- Other Stakeholders: other stakeholders include production and business associations, universities and research institutes, chambers of commerce, private consultants and advisors, sanitary and epidemiological services, and certification authorities.

2.3. Sub-regional key differences

Arab countries share several commonalities in different parts of the production, post-production and marketing of the date value chain. Yet, there are clear features that are specific to countries which are often also shared by other countries in their respective sub-regions. In the context of date production, these sub-regions can be divided as Gulf Countries (GCC countries plus Iraq), Mashreq, Maghreb, and the Nile Valley countries.

Date palm in the Gulf sub-region: the date farming systems in GCC countries are mostly traditional (small-scale orchards of date trees based on labor intensive techniques with shortage of labor) with modern plantation in UAE and KSA. Also, intercropping system is practiced with other crops. About 277,000 hectares was under dates cultivation in GCC countries in 2016, however high variability characterizes this sub-region in terms of areas cultivated, volumes of production and yields (see Tables 2 and 3). The date production in this sub-region can be considered new compared to other sub-regions.

Date palm in the Mashreq sub-region: Date palm production in Mashreq sub-region countries represents a source of income and nutrition to oasis inhabitants and it contributes to providing job opportunities for many rural women. The date farming systems in this sub-region countries are mostly traditional (Small scale orchards of date trees depending on labor for the cultural practices) with some modern plantations. High variability also characterizes the Mashreq sub-region as regarding the areas cultivated by date palm and the average date production per country. The main cultivars planted in the sub-region are Khastawi, Barhee, Halawy, Dayri, Hayany and Khadrawy, Majdool, Zahdi and Sayer.

Date palm in the Maghreb sub-region: the date farming systems are mostly traditional take place in oases through mostly small-scale orchards and some modern plantations which were newly introduced. Both systems practice intercropping. The area cultivated by date palms in Maghreb sub-region countries was 64,700 ha in 2016. Average date production in the countries of the Maghreb sub-region was about 318,000 tons in 2016, with high variability (the highest in Algeria and lowest Mauritania (see Figure 1). Tunisian date palm plantations are characterized by the prevalence of Deglet Noor variety in spite of their large genetic diversity. It occupies approximately 60% of the Tunisian palm plantations and continues to be multiplied (Bouguedoura, Bennaceur et al., 2015). In Libya the main cultivars are Bukerary, Taboni, Lamsy, Blonde, Halaway, Bronzi, and Baudi while the Algerian palm groves currently have 45% of Deglet Noor (Bouguedoura, Bennaceur et al., 2015). The Moroccan date is characterized by the existence of a host of varieties, with a predominance of so-called “Khalt” varieties (unidentified), which have low commercial value. Commercial varieties (Deglet Noor and Mejdoul) are produced and exported to European markets which premium high-quality products.

Date palm production in the Nile Valley sub-region: the date farming systems are mostly traditional with some modern plantations and intercropping systems. The area cultivated by date palm in 2016 was 48,000 ha in Egypt and 37,000 ha in Sudan. In Egypt, date palm is cultivated and grown wherever water is available and the date industry supports over one million people. 'Hayany' is the cultivar most planted, but the best varieties suitable for marketing at the khalal stage are Zaghloul dates, which are the most economically important in Egypt (Kassem, 2012). The date production in Sudan totaled 423,662 tons from an area of 36,545 ha in 2016. About 98% of the dates grown in Sudan are either cultivar classed as dry, or semi-dry. Date marketing in this sub-region is mostly domestic with limited export of table dates because of limited processing capacity.

3. Dates end-markets

The types of local and export end-markets that date producers/traders/processors in the region can reach directly affects the prices that dates can be sold at, as well as the levels of profitability and performance of the entire date value chain. This is especially important for export markets which offer a wide range of prices and quality requirements. The quantities of dates imported by the main date trade partners to the Arab countries has been fluctuating in the last years (2012-2016), but India clearly ranks first followed by UAE, Morocco and France (see Table 2). The dates that are imported by the Asian importing countries are table dates, which are usually marketed unprocessed, while that for EU countries are mostly processed dates. Notably, as indicated in the Table 2, there is an important regional trade of dates among countries in the region, as is the case of UAE, Morocco and Oman which are date producing countries but whose levels of date imports are very substantial to satisfy un-met local demand. In the case of Oman and UAE the imports are usually to satisfy the requirements of certain class of consumers or for re-exporting after adding value through proper packing and/or processing.

Table 2. Annual imports of main date importing countries (tons)
Source: Authors based on UN COMTRADE international trade statistics

According to data from UNCOMTRADE (2018), the volume of dates exports from the Arab countries, UAE ranked first, followed by Tunisia, KSA and Egypt in the last quinquennial (see Table 3). Although UAE dates exports declined with a negative 2% annual rate during the period 2012-2016, in comparison with other major countries in the region and even with other world exporting countries such as Iran, Israel and Pakistan, it has ranked first and achieved the highest record of dates export. The average yearly export from UAE in about 295,000 tons (Figure 3). The date exports from the KSA, (mostly table dates) has shown increasing trend with annual average growth of 20% in the last five years. The Tunisian date export, which is of high quality (Deglet Nour variety), has been slightly growing during the same period with a 4% annual average growth rate. While smaller in volume than the exports of UAE and KSA, Tunisia is a key export country in the region because of its volumes but also due to its high quality of dates.

Table 3. Volume of dates exports from main exporting countries and average export prices
Source: Authors based on UN COMTRADE international trade statistics

Countries that have succeeded in expanding exports have invested in a conducive enabling business environment. Tunisia is a good showcase as it has a solid commercial dates industry, which is the result of a production system which cares about market requirements, and well-established marketing institutions which are operational on setting standards according international demand and targeting of certain markets that have special requirements. In the case of countries like KSA, the industry is yet to be more organized and the commercialization of the date value chain is still recent.

Date exporting prices have greatly varied between the main world exporting countries according to the trends in volumes of production and reflecting the types of markets that the country can access according to the quality of their dates. An aspect which stands out in Table 3 is the fact that the average price received by Egypt, Tunisia and KSA are substantially higher than that of other Arab countries like UAE and Iraq, and even higher than other countries out of the Arab region such as Iran and Pakistan. This seems to underscore the ability of these countries to access higher value date markets, such as the EU one, which premiums higher quality dates. The rest of the countries in the region are exporting to lower value markets in countries like India, Bangladesh and some countries in the NENA region.

As indicated in the Figure 3, major dates exporting countries trade their dates to a number of countries, with India absorbing more that 63% of the exports from UAE, Morocco more than 29% of exports from Tunisia, Turkey more than 36% of exports from KSA, while KSA imported more than 29% of exports from Egypt. The UAE is a major importer of dates, accounting for more than 24%, 14% and 10% of dates sourced from Iran, KSA and Egypt, respectively. The EU market, which offers a great opportunity for countries in the region to increase the value of exports, has only been explored by Tunisia. This seems to explain while the average price of exports of dates from Tunisia is higher than most countries in the Arab region. The fact that India takes almost two thirds of the exports from UAE also seems to correlate with the low average prices that the UAE gets on their exports.

In summary, there are significant marketing opportunities for dates in the local markets, in dates importing countries in the region, and in the world market. These markets, especially the export one, are constantly expanding and increasingly becoming a pull force for countries in the region to address their internal and regional issues which affect the productivity, quality, costs and access to demanding requirements in the international markets. There are great opportunities in high value markets such as Australia, EU and Canada, which could represent a rapid increase in the value of dates exported from the region. Likewise, other less demanding lower quality markets (India, Indonesia, Bangladesh, etc.) could also help to expand the volumes of dates exports from the region, especially from countries like Algeria and Iraq.

4. Systemic Constraints and Upgrading Opportunities

Increased investment and adoption of modern date palm technologies such as tissue-cultured date palms have led to further expansion of healthier and more productive date orchards in many parts of the world, particularly in the NENA region. However, as suggested by Manickavasagan, Essa et al. (2012) most date plantations still lack modern and appropriate solutions to issues related to cultural practices, such as hand pollination; seedlings development; water and soil fertility management; harvesting timing and methods; postharvest handling; and by-product development and utilization; transport and storage. Several studies have addressed these bottlenecks and report improved yields and fruit quality by following proper production and post-harvest management in dates cultivations (Al-Yahyai & Al-Kharusi, 2012; El Mardi, Al Julanda Al Said et al., 2006). Pest and disease management of date palm also needs considerable research attention. The most common pests of date palm in the Arab region are Dubas bug and Red Palm Weevil. Chemical control of these pests has had limited success, however, a broader ban on pesticides and increased demand for chemical-free dates require alternative and probably more integrated methods of pest management.

While significant, the constraints that affect the date palm value chain face in the Arab region are not limited to production. Along different functions of the value chain there are key factors that affect the ability of the countries to increase profitability of dates production, and improve post-harvest, processing and marketing practices. The expansion of the date palm industry in the region is dependent on addressing these issues related to core functions of the value chain but also other problems related to business enabling environment, horizontal and vertical linkages, and supporting services as described in the Table 4.

Table 4. Key constraints and opportunities for expanding the date value chain in Arab countries
Value Chain Function Main Constraints Opportunities
Input Supply and Demand - Non-market oriented varieties (low productivity and low quality)

- Limited economic/physical access to inputs
- Shortage and high cost of labor
- Limited access to equipment for production, harvest and post-harvest - Introduction of new date varieties
- Public and private sector extension can raise awareness about fertilizers and other inputs/technologies
- Collecting centers for mechanization of joint post-production activities

Production - Poor farm management and adoption of cultivation practices

- Small sized farms and aged trees
- Low quality of date varieties
- Poor management of pest and diseases (IPM)
- The spreading of Bayoudh disease and RPW has impoverished the ancient date palm heritage Shortage of qualified and trained labor
- Inadequate access to agricultural credit
- Low profitability makes traditional groves in oasis unattractive -Improve horizontal linkages between farmers for economies of scale to increase access and reduce costs
- Introduce mechanization to increase labor productivity
- Land reclamation programs
- Introduction of high yield date varieties
- Promotion of IPM to address key pests and diseases threats
- Improve quarantine, surveillance and monitoring of pests and diseases
- Expand finance to date producers

Processing - Poor postharvest practices

- Field and post-harvest losses are high
- Limited options of processing plants that can produce a variety of products
- Poor relationship between the farms and date processing factories
- Poor exploitation of facilities/infrastructure to enhance production and marketing of dates
- Low diversification of date products - Acquaint farmers with date post-harvest and processing practices
- Incentivize the establishment of more dates processing plants (small and large scale)
- Diversify dates products and by-products

Wholesaling & Retailing - Limited linkages with small-scale producers

- Poor handling and packing
- Low storage capacity - Improve linkages with small-scale farmers through collectors
- Introduce equipment for handling of dates
- Establishment of storage facilities

Output Market - Countries constrained to meet stringent market requirements (food quality and safety; varieties)

- Limited availability of market information

- Stiff competitiveness in external markets - Invest on improving processing and packing of dates products
- Promote awareness along the value chain about quality and standards
- Increase economic efficiencies along the value chain
- Supporting Services - Absence of internationally accepted standards for marketing dates
- Poor local food safety and quality control systems affect countries capacity to reach high value markets
- Limited regional coordination to deal with IPM issues
- Insufficient research and development - Adoption of quality standards in date trade
- Strengthening of national food control systems and traceability
- Development of regional and sub-regional coordination for pest and disease control

Business Enabling Environment - Absence of date sector development policies

- Poor transportation and post-harvest infrastructure (roads, cold facilities, storage)
- Weak marketing institutions
- Limited financing to traditional producers
- Security and political unrest in some countries
- Limit presence of business support services - Strategic infrastructure investment projects
- Establishment and strengthening of marketing institutions
- Promotion of traditional and non-traditional credit schemes
- Mainstreaming the importance of the sector with policy/decision makers
- Source: Authors

5. A strategy for improved competitiveness of the date palm value chain

Given the important problems that affect dates producing countries in the region and the significant marketing opportunities both internally and in importing countries, it is paramount to setup a strategy for increasing the competitiveness, expand profitability, and growth of the dates value chain. This can be a pulling force to concertedly find sustainable business-driven solutions to the main constraints described above. This strategy must be stakeholders' driven; clearly identify the vision on how to increase competitiveness and the marketing strategy (price leadership, quality leadership, and/or differentiation); what needs to happen to achieve real systemic impact in competitiveness; and where to focus investments (prioritize actions).

This strategy in being defined in the exercise that AOAD and FAO are currently conducting in the region, which through the conduction of a comprehensive regional value chain study (including country studies) is developing a strategic framework for the development of the date value chain in the region. This strategy focus on addressing key bottlenecks, investing in leverage points and upgrading; is business-driven; and aims at fostering win-win relationships for value chain stakeholders. The framework also recognizes the differences that the countries in the region have in terms of the most suitable strategy to achieve their growth goals. Countries like KSA and Iraq can compete on price leadership, while Algeria and Tunisia can continue to expand their production of high value varieties to compete on quality leadership in high value markets.

6. CONCLUSION

Date palm represents an especial icon in agriculture in the NENA region. Since its origin is believed to be in the Arab region, dates have a unique historical, religious, economic, and social value. With an estimated production of nearly eight million tons in 2012, dates represent a main source of income and staple food for virtually all countries in the region. The rich nutritional composition of dates, which includes carbohydrates, vitamins, and protein, also highlights its importance to food security and nutrition. There is significant marketing opportunities for commercializing dates in the local markets, to dates importing countries in the region, and in the world market. These markets, especially the export one, are constantly expanding and are increasingly a pull force for countries in the region to address their internal and regional issues that affect the productivity, quality, costs and access to demanding requirements in the international markets. There are great opportunities in high value markets such as Australia, EU countries, and Canada, which could represent a rapid increase in the value of dates exports from the region. Likewise, other less demanding lower quality markets (India, Indonesia, Bangladesh, etc.) could also help to expand the volumes of dates exports from the region, especially from countries like Algeria, KSA, and Iraq.

Despite these potentials and significant recent efforts, the date sub-sector still has an enormous room to grow in the region, and efforts should focus on integrated national and regional strategies. The expansion of the date palm sub-sector is dependent on controlling the spread of RPW and other pests and diseases, which continues to have a negative impact on productivity and quality; but also enhancing the quality of date products; reducing field and post-harvest losses; formalizing and streamlining market systems; and, developing date value added products and byproducts. This paper attempts to propose a more holistic, market-driven and inclusive approach to sustainably develop the date value chain in the region and at the country level, with a focus on increasing competitiveness, access to higher value markets, and expanding growth.

The paper also highlights systemic constraints and its opportunities related to core functions along the value chain, the enabling environment, horizontal and vertical linkages, and supporting services. Most importantly, it elaborates around concerted regional and country level strategic integrated efforts needed to address systemic bottlenecks in the date palm value chain. These efforts are analyzed through the lens of a wide value chain and food system approach and include: introduction of new high yield and disease resistant date varieties; public and private sector extension to raise awareness about the use fertilizers and other technologies; establishing collecting centers for mechanization of joint post-production activities; improve horizontal linkages between farmers for economies of scale to increase access and reduce costs; land reclamation programs to expand farm sizes; promotion of IPM to address key pests and diseases threats; improve quarantine, surveillance and monitoring of pests and diseases; expand finance to date producers; diversify dates products and by-products; strengthen national food control systems and traceability; increase economic efficiencies along the value chain; develop regional and sub-regional coordination for pest and disease control; promotion of traditional and non-traditional credit schemes; and mainstreaming the importance of the sector with policy/decision makers.

Bibliography

Al-Khayri, J. M., Jain, S. M., & Johnson, D. V. (2015). Date Palm Genetic Resourse and Utilization: Springer.

Al-Yahyai, R., & Al-Kharusi, L. (2012). Physical and chemical quality attributes of freeze-stored dates. Int. J. Agric. Biol, 14(1), 97-100.

Bouguedoura, N., Bennaceur, M., Babahani, S., & Benziouche, S. E. (2015). Date palm status and perspective in Algeria Date palm genetic resources and utilization (pp. 125-168): Springer.

El Mardi, M. O., Al Julanda Al Said, F., Bakheit Sakit, C., Al Kharusi, L. M., Al Rahbi, I. N., & Al Mahrazi, K. (2006). Effect of pollination method, fertilizer and mulch treatments on the physical and chemical characteristics of date palm (Phoenix dactylifera) fruit I: Physical characteristics. Paper presented at the III International Date Palm Conference 736.

FAO. (2016). FAOSTAT Database Collections. Retrieved 2016 http://faostat.fao.org

FAO. (2018). FAOSTAT Database Collections. Retrieved 2018 http://faostat.fao.org

Kassem, H. (2012). The response of date palm to calcareous soil fertilisation. Journal of soil science and plant nutrition, 12(1), 45-58.

Liu, P. (2003). The marketing potential of date palm fruits in the European market. Raw Materials, Tropical and Horticultural Products Service Commodities and Trade Division. FAO, Rome.

Manickavasagan, A., Essa, M. M., & Sukumar, E. (2012). Dates: production, processing, food, and medicinal values: CRC Press.

Sawaya, W. N. (2000). Regional Network for Date-Palm in the Near East and North Africa. AARINENA. Retrieved from http://www.fao.org/docs/eims/upload/211145/Date_Palm_Proposal.pdf

UNCOMTRADE. (2018). International Trade Statistics - Import/Export Data. Retrieved from: https://comtrade.un.org

Impact of the weather conditions on the date palm in Al Qassim region, Kingdom of Saudi Arabia

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Abstract

A field study was conducted to survey the damage of frost on some date palm varieties in Al-Qassim region in Saudi Arabia where the region was exposed to three waves of frost in January 2008, February 2016 and February 2017, where the air temperature was decreased to -4, -2 and -2, respectively and the exposure time were 8, 5 and 5 hours per event, respectively. Where the study was conducted in Al-Batin project in Al-Qassim region, Saudi Arabia (26° 15^{-} E - 44° 9^{-} N). The study evaluated the damages were induced by the frost on the Vegetative parts (the number of damaged fronds per tree), the reproductive parts (number of damaged spathes per tree), the percent of the immature fruits (khlal) and date palm tree productivity, Kg/tree on 15 of main varieties of dates in the region; Sukkary, Khalas, Khodry, Sagae, Hashishi, Nabut Saif, Nabut Ali, Rashudy, Barhi, Rothanah, Burimi, Rezizi, Shaqra, Maktoumi, Male trees. The results showed that the greater damage were on the vegetative and reproductive parts by decreasing the air temperature below than 0° with increasing in the number of hours of frost exposure, where the first year (2008) was the most harmful to the palm while the third year (2017) is the least harmful. The results indicated that the most of the studied varieties were affected by frost at temperatures dropping to -2 with 5 hours' exposure duration, where the number of affected fronds was 1-12 fronds/tree except the varieties of Nabut Ali, Maktoumi and Rezizi were the most tolerant varieties for frost. In addition, the vegetative parts of the trees of Khalas variety is considered the most sensitive for frost which was affected by an average of 12, 8 and 8 fronds/tree at 2008, 2016 and 2017, respectively. The early flowering varieties (Sukkary, Hashishi and male trees) were the most affected by frost, where the number of damaged spathes was in average of 0, 3, and 3 in 2008, 2016 and 2017, respectively. The most affected variety by frost was Sukkary where the damages were represented in spathe atrophy, didn't split opened and the most of them infected by fungi. The results showed also that there was a decrease in the productivity of affected date palm trees by the frost, where the production was decreased by 65%, 40% and 35% for 2008, 2016 and 2017, respectively for Khalas variety which was the most affected by frost. It could be concluded that the frost has a severe economic damage on date palm trees, which were varied with the amount of decrease in air temperature degrees, as well as the number of hours of decline in temperature and it should be predicted and take precautions before it occurs.

Socioeconomic Analysis of Date Palm Sector: The Case of Biskra Region (Algeria)

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Abstract

Regarding the importance of date palm sector for local development, this study aims to explore and analyze empirically the main socioeconomic characteristics of date palm sector in Biskra region. The study uses an extensive micro-database containing 21,502 date palm farms. The economic aspect of date palm farm is reflected by two structural features: size and tree density; since the social aspect is reflected by human capital dimension measured by farmer's age and his experience. Our study is mainly explorative and attempts to extract the empirical regularities which would be helpful for policy making.

Keywords: Date Palm, Farm Size, Density, Human Capital, Biskra Region

1. INTRODUCTION

The date palm sector gains an increasing importance for the national economy in Algeria. Besides, it has an increasing interest in terms of exportation, subsidies and promotion last years. Nowadays, Algeria has a total cultivated area for date palm of 162 372 ha (with a total number of palm trees roughly 18 millions). We mention also that Algerian date palm sector includes about 800 date palm cultivars[1].

It recognized that the Wilaya of Biskra occupy the first place at the national level for date palm farming. This Wilaya (known also as Zibans Region) is the focus of our study. From our data, it has a total cultivated area of 124 826 ha, i.e., it represents about 76% of the total date palm cultivated area in Algeria. Also, date palm sector in Biskra region contains 3.5 millions of palm trees representing nearly 30% of national sector. This confirms the fact that date palm sector in Biskra Region has a considerable importance in the national economy.
Our study aims to explore and analyze the main socioeconomic characteristics of date palm sector in Biskra region through an extensive micro-database containing 21,502 farms. The date palm farm is the unit of study. The economic component of date palm farm is reflected by two structural features: size and tree density. Since the social component includes the human capital by farmer's age and experience. Our study is mainly explorative and attempts to extract the empirical regularities which would be helpful for policy making.

The paper is organized as follow. The next section describes the research methodology. Section 3 analyzes the economic aspect of date palm sector, since section 4 focuses on its social dimension. Section 5 concludes.

[1] These statistics are provided from the studies of Benziouch (2013) and Bouguedoura et al. (2015)

2. RESEARCH METHODOLOGY

The study uses an extensive micro-database collected by the Algerian Ministry of Agriculture (Department of Extension Services, Biskra). The database concerns the date palm farming sector in Biskra region. It contains 21,502 observations as date palm farms. Several measures were captured. The first reflects the farm size expressed in terms of the effectively irrigated and cultivated area of the date palm farm (in hectares). Another measure is captured which reflects a technological aspect of date production. It concerns the technical density for date palm farms expressed in terms of palm trees *per* one hectare. Other measures were also included in the data such as whether the farm is specialized or diversified; and the total number of palm tree in the farm. For the socioeconomic measures, the data we have captures two. It concerns the farmer's age (in years) and the farmer's experience in date palm farming.

For the purpose of the study, we employ some basic statistical tools, namely, the statistics of central trend (mean, median and mode), the dispersion (standard deviation), two-dimensional plots, and frequency distributions. The later include the relative frequencies distribution (with adequate number of bins) and the non-parametric kernel density estimation. Kernel density estimation proceeds by defining a set of evenly spaced reference points, and attributes a weighted sum of the data frequencies in the neighborhood of the point being estimated. The formula used to compute the estimated density at each reference point, *x*, is

$$f(x) = \frac{1}{nh}\sum k(\frac{x - x_t}{h})$$

where *n* denotes the number of data points, *h* is a bandwidth parameter, and *k*(.) is the kernel function[2]. For facilitating the analysis, the densities presented in this study were estimated using the same bandwidth of 2.0[3].

3. ECONOMIC ANALYSIS OF DATE PALM SECTOR

In this section, we focus on the economic aspect of date palm farm by analyzing its main structural characteristics, namely, farm size and tree density. Farm size, according to cropping pattern here[4], correspond to the cultivated are (in hectares), where the technical density in date palm farms express the number of date palm trees in one hectare reflecting the main component of date palm technology farming.

3.1. Farm Size

Table 1 summarizes the main statistics of date palm farm size in Biskra region. From the data we have (with 21 502 pal date producers), it seems that the date palm farm size has an average of 6.68 ha (having a minimum value of 0.09 ha and a maximum value of 906 ha) with a low standard deviation (13.38).

From the side of the median and the mode, they represent respectively 4 ha and 1 ha. The significant differences between these basic central moments require a more deep investigation for the date palm farm size dimension. In order to analyze this dimension in more detailed terms, we have split the farm size in three classes. The first represents the small size

[2] Where the larger the value of the bandwidth parameter (the width of the neighborhood), the smoother the estimated density, i.e., varying the bandwidth parameter allows for control of the degree of smoothing in the estimated density (Silverman, 1986).

[3] Estimation with different bandwidths did not produce qualitatively different results.

[4] To the extent that the farm size is a complex issue in empirical literature. See Eastwood *et al.* (2010) for more details on this matter.

farms (farm area less than 5 ha), the second for the medium size farms (farm area between 5 and 10 ha), and the last one is for the large size farms (farm area more than 10 ha).
As shown in Table 1, frequencies of different size classes are as follows: Small date palm farms represent roughly 54% (i.e., 11 646 farms), whereas the medium size corresponds to 22.5% (i.e., 4 835 farms), since larger farms has a fraction of 23.5% (i.e., 5 021 farms). It seems that the small date palm farm has the important share in date palm sector of Biskra region.

In more detailed terms, small farms have an average of 2.13 ha with median value of 2 ha (having low standard deviation: 1.16). Since the medium farms have an average of 6.2 ha with median value of 6 ha (having low standard deviation: 1.37), meanwhile large farms have an average of 17.66 ha with median value of 12 ha (having high standard deviation: 24.34).

In order to shows more accurately the modality structure of date palm farm size, we proceed results of the kernel density estimation. The results are shown in the following figures. Figure 1 shows the plot of the estimated frequency distribution for farm size which are less than 5 ha, since the Figure 2 shows plot of the estimated frequency distribution for farm size which are more than 5 ha[5].

From these tow figures, the multimodality of farm size is obviously observed. The first model value is about 1 ha (more exactly from 1 to 1.5 ha), the second modal value is 5 ha (more exactly from 4.5 to 5 ha) and the third one is exactly 10 ha. The shape of large farm distribution in Figure 2 is justified by the higher value of the standard deviation. We mention that there is just 27 observations beyond the size of 100 ha, and 940 observations between 20-100 ha. Hence, by using the remaining significant observations (i.e. 20 536 farms), we proceed a frequency distribution plot (with 10 bins). The result is shown in Figure 3 below.

From Figure 3, we can observe that the small farms (less than 5 ha) have the higher frequencies in date palm sector, since the higher frequency beyond that (i.e., large farms) is for the size of 10 ha. The main finding from this explorative analysis is that the date palm farm size in Biskra region exhibits a bimodal distribution with the dominance of small farms.

3.2. Technical Density

From the plantation density in date palm sector, expressed by the number of date palm trees in one hectare, Table 2 displays the main statistics of date palm tree density in Biskra region. From the extensive data we have, it seems that the date palm plantation density has an approximate average of 46 tree/ha (having a minimum value of 0.05 tree/ha and a maximum value of 437 tree/ha) with a high standard deviation (46.71).
The divergent value of the mode for the overall data implies further detailed investigation. It seems that, from our data, there are just 190 observations having values higher than 200 palm tree/ha. Then, we proceed a frequency distribution plot (with 200 bins). The result is shown in Figure 4.

The result form Figure 4 shows that the higher frequencies for the technical density for date palm farms are respectively for less than 20 tree/ha and exactly 100 tree/ha. The first modality could reflect lower densities as a typical characteristic of modern date palm farming system. As for the second modality, reflecting higher typical density of 100 tree/ha, it could be explained by the presence of highly specialized and traditional date palm farming system. In order to investigate the relationship between this measure and the size dimension (analyzed above), we plot the two measures in two-dimensional graphic as shown in Figure 5 below.

It obvious from the resulted plot that there is an empirical regularity between date palm farm size and palm tree plantation density. A decreased non-linear slope could be visibly

[5] This classification seems to give more visibility for the multiplicity of modal values in the data.

detected. Larger farms tend to have lower densities, where small farms have higher ones. These tendencies could be explained also by the dual nature of date palm farming systems. Larger farm is the main attribute of traditional system since smaller farm is an attribute of modernized and newest farms.

4. HUMAN CAPITAL IN DATE PALM SECTOR

The social aspect will be the focus of this section. The study highlights the farmer's age and its experience as proxies of the human capital[6] in date palm sector. These two measures will be analyzed mainly in terms of farm size, density, and specialization.

4.1. Age Structure

In order to analyze the date palm farming sector in terms of farmers' age structure, we have proceed a plot for frequency distribution of date palm producers' age (with 3 bins). The result is shown in Figure 6 below.

The plot in Figure 6 shows three distinguished age classes. The first class represents farmers having less than 40 years old including 3 137 farms (14.5%). The second class is between 40 and 80 years old including 16 019 farms (74.5%), since the third one include older farmers (i.e., more than 80 years old) including 2 346 farms (11%). The overall average of date palm producer's age is about 57 years (with median and modal values of 56 years and 47 years respectively).

The Table 3 shows the main statistics of farmer's age classes. The first age class has the following characteristics: A mean age of 34 years, a mean farm size of 6.43 ha, a mean total trees number of 195 palm trees, a mean density of 49 palm tree/ha, 77% of them are specialized in date palm farming, and they have in average 16.6 years of experience.

Besides, the second age class has the following characteristics: A mean age of 57 years, a mean farm size of 6.63 ha, a mean total trees number of 157 palm trees, a mean density of 46 palm tree/ha, 68% of them are specialized in date palm farming, and they have 14.6 years average of experience. Since, the third age class has the following characteristics: A mean age of 85 years, a mean farm size of 7.1 ha, a mean total trees number of 140 palm trees, a mean density of 45 palm tree/ha, 67% of them are specialized in date palm farming, and they have 16.4 years average of experience.

These findings allow us to conclude that date palm farming is dominated by the second age class by having an intermediate values for the underlying attributes. More interestingly, younger farmers (the first age class) have smaller size, higher density and they are the most specialized compared to the others, whereas conversely, older farmers (the third age class) have larger size, lower density and they are the less specialized.

4.2. Human Capital

We aim now to analyze the human capital aspect, as reflected by the years of experience in date palm farming. In order to do so, firstly, we have proceed a kernel frequency distribution of date palm producers' experience. The result is shown in Figure 7 below.

The Figure 7 allows us to distinguish obviously two classes: The first class for the less experienced farmers (less than 10 years) with a share of 18%, and a second class for the more experienced farmers (more than 10 years) with a share of 82%. Therefore, more detailed investigation is required. Table 4 summarizes the main statistics of farmer's experience in terms of two classes.

[6] See Huffman (2001) for more details on human capital in agriculture

The first class (less experienced) has the following characteristics: A mean experience of 6 years, a mean farm size of 6 ha, a mean total trees number of 149 palm trees, a mean density of 57 palm tree/ha, 68% of them are specialized in date palm farming, and they have 61 years old in average.

Since the second class (more experienced) has the following characteristics: A mean experience of 16 years, a mean farm size of 7 ha, a mean total trees number of 163 palm trees, a mean density of 44 palm tree/ha, 70% of them are specialized in date palm farming, and they have a mean of 56 years old.

These findings suggest the following: date palm farming is dominated by more experienced old farmers with medium size, lower density and they are more specialized compared to the class of the less experienced. The analysis of this aspect could have important implications on the knowledge transfer between the two generations present clearly here.

4. CONCLUSION

The aim of this study was to explore and analyze the main socioeconomic characteristics of date palm sector in Biskra region through an extensive micro-database containing 21,502 farms. By using some analytical tools, the findings of this study can be summarized as follow: In date palm farming of Biskra region, the average farm size is about 6.6 ha having a bimodal distribution, the averaged density is about 46 trees/ha, younger farmer has smaller size, higher density and they are the most specialized, there is a dominance of more experienced old farmers with medium size and lower density.

The present study should be extended by examining the potential drivers of the date palm farm efficiency, with a particular emphasis on the potential role of land regulations, subsidies and agricultural policies in date palm sector. More accurate analysis would contribute to the debate on the efficiency of a public intervention in date palm sector to promote the modernization of farming, particularly oriented toward a specific model. However, this research is still a useful exercise in itself, since it lays the framework for subsequent future testing and refinement.

REFERENCES

Benziouche, S.E. 2013. The Sector of Dates in Algeria, Role in National Economy and Position on the International Market. *Acta Horticulturae*, 994: 155-162.

Bouguedoura, N., Bennaceur, M., Babahani, S. and Benziouche, S.E. 2015. Date Palm Status and Perspective in Algeria. In: *Date Palm Genetic Resources and Utilization*. Springer Netherlands. 125-168.

Eastwood, R., Lipton, M. and Newell, A. 2010. Farm Size. In: *Handbook of Agricultural Economics.* Elsevier Science. 3323- 3397.

Huffman, W.E. 2001. Human Capital: Education and Agriculture. In: *Handbook of Agricultural Economics.* Elsevier Science. 334-381.

Silverman, B.W. 1986. *Density Estimation for Statistics and Data Analysis*. CRC press.

Tables

Table 1. Summary Statistics of the Date palm Farm Size

	Obs.	*Mean*	*Median*	*Mode*	*Min*	*Max*	*S.D.*
Farm Size (ha)	21 502	6.68	4	1	0.09	906	13.38
Less 5 ha	11 646	2.13	2	1	0.09	4.99	1.16
From 5 to 10 ha	4 835	6.20	6	5	5	9.99	1.37
More 10 ha	5 021	17.66	12	10	10	906	24.34

Table 2. Summary Statistics of the Date palm Tree Density

	Obs.	*Mean*	*Median*	*Mode*	*Min*	*Max*	*S.D.*
Tree Density	21 502	46.47	30	100	0.05	437.50	46.71
Less than 50 palm/ha	14 413	19.91	16	50	0.05	50.99	13.82
From 50 to 100 palm/ha	3 744	72.03	70	60	51	99.83	12.81
More than 100 palm/ha	3 345	132.30	120	100	100	437.50	43.37

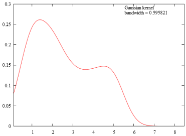
Table 3. Summary Statistics of Farmer's Age Classes

Classes	*Obs.*	*Mean Age*	*Farm Size*	*Palms Number*	*Tree Density*	*Specialized Farms (%)*	*Farmer's Experience*
Age Class 1	3 137	34.26	6.43	194.96	49.05	77	13.63
Age Class 2	16 019	57.32	6.63	157.04	46.13	68	14.60
Age Class 3	2 346	85.48	7.10	140.76	45.31	67	16.49

Table 4. Summary Statistics of Farmer's Experience

Classes	*Obs.*	*Farmer's Experience*	*Farm Size*	*Palms Number*	*Tree Density*	*Specialized Farms (%)*	*Mean Age*
Less than 10 years	3 815	6,12	5,99	148,85	57,08	68	61,64
More than 10 years	17 687	16,64	6,82	163,38	44,19	70	56,04

Figures



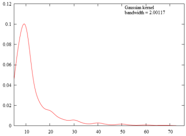


Figure 1. Estimated Frequency Distribution for Farm Size (Less than 5 ha)

Figure 2. Estimated Frequency Distribution for Farm Size (More than 5 ha)

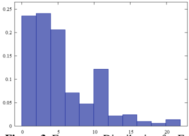


Figure 3. Frequency Distribution for Farm Size (Less than 20 ha)

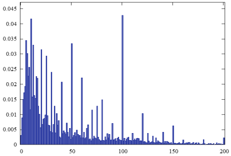


Figure 4. Estimated Frequency Distribution for Palm Tree Density

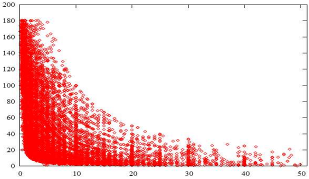


Figure 5. Palm Tree Density in Terms of Farm Size

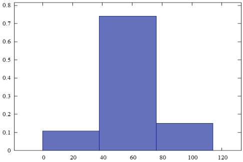


Figure 6. Frequency Distribution for Palm Date Producer's Age

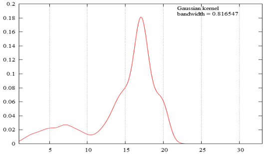


Figure 7. Estimated Frequency Distribution for Farmers' Experience

Stable carbon and nitrogen isotope signature and vegetation indices as indicators of date palm performance under salinity

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Abstract

Date palm is frequently cultivated under irrigation with brackish water, which may affect their growth and productivity. Developing monitoring techniques on the effect of salinity on this species may be relevant either in agronomy, informing on the effect of different salinity levels, or for breeding, as a phenotyping tool to select more tolerant genotypes. A set of 16 different elite date palm varieties from U.A.E. and K.S.A. were grown for 15 years under irrigation with three different levels of saline water (5, 10 and 15 dS m^{-1}) at the International Center for Biosaline Agriculture (ICBA) located in the UAE. A range of different vegetation indices, informing on the degree of canopy greenness, were calculated from single tree-top images taken with an RGB camera and using the open-source software Breedpix. In addition, canopy temperature (CT) was measured, as indicator of water status, with an infrared thermometer. Further the carbon isotope composition ($\delta^{13}C$), as time-integrated indicator of water status, and the nitrogen isotope composition ($\delta^{15}N$) and the total nitrogen content (N), were measured with a mass spectrometer and an elemental analyser, in the dry matter of fully developed leaflets and date stones. Irrigation conditions and genotypes exhibited significant effect for all the remote sensing and stable isotope traits evaluated. When the three salinity levels and the set of varieties were combined $\delta^{13}C$ and CT correlated negatively with growth parameters (trunk height and diameter and number of branches) but the vegetation indices correlated even stronger. However, correlations across varieties within a given salinity level were in general weaker but in most cases still significant and N and $\delta^{15}N$ correlated positively with fruit yield and growth within the extreme salinity levels. Moreover, the combination of few traits of different categories (vegetation indices, stable isotopes or N) allowed to predict for most of the growing conditions at least a quarter of the genotypic variability in growth and productivity. In addition, the combination of these different traits through a path analysis allow to draw single empirical models that illustrate the main genotypic physiological features associated with a better performance in terms of growth and productivity within each salinity level.

Economic Efficiency of Innovative Investment in Date Palm Sector: The Case of the Sultanate of Oman

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Abstract

The paper reviews the economic importance of date palm sector, the role of innovative investment based on sound economic fundamentals and technical upgrading of the sector both in terms of economic and developmental impact; increase its contribution to GDP, diversification and increased employment optimizing resources and sustainability.

The paper is based on the technical and economic feasibility studies and practical examples of leading models of Oman, and other Arab countries, during the past years, and cite the development projects financed by the Agricultural and Fisheries Development Fund (the second place winner, for best development project, of Khalifa International Date Palm Award 2011, the pilot prize and its renewable thinking and practices), in addition to other forms of positive economic and environmental impact.

The paper presents recommendations and proposals based on the strategic directions of the Sultanate of Oman in the agriculture sector in particular, as well as keep up with the expectations of the outcomes of the fourth industrial revolution could be used for further development, sector performance continued in its contribution to sustainable agricultural development, support small and medium-sized enterprises and youth entrepreneurship to achieve the objectives of food security of Arab states.

INTRODUCTION

The Sultanate of Oman has witnessed remarkable development goals for the last four decades. Started the second halve of the 1970's Oman embarking economic, socioeconomic and political improvements via five years' development plans. A significant milestone was, the adaption of Oman Vision 2020. Since 1995 Oman set major goals to develop its human resources and diversify the economy to reduce dependency on Oil. Transportation and communication infrastructure, tourism, agriculture and fisheries, modern information technology, logistics, and other non-oil industries have witnessed major promotion and improvements.

Agriculture

The Vision 2020 envisages that agriculture and fishery's share of GDP would rise to 5.1 % in 2020, from about 2 % in 2000. Agriculture and fisheries are of vital sectors of the national economy in terms of supporting active contribution to diversify income sources, and promote non-oil GDP, employment, social stability and enhancing food security in the country. The total area of the Sultanate (309.5) thousand square kilometres, (5.5) million feddan arable, whereas agricultural land holdings (355) thousand feddan, with good livestock wealth, (3.4) million heads. Omani coast about (3165) kilometres, biomass stock estimated of some (7.6) million tonnes, as exploitable quantity amounts to approximately (2.3) million tonnes, (30%), while the amount of exploited fish production by the end of 2016 was (280) thousand tonnes (12%) of total exploitable quantity.

The MoAF of Oman, in collaboration with FAO experts, have developed a Sustainable Agricultural and Rural Development Strategy (SARDS) until 2040. The aim is to bring about a significant improvement in the country's food and nutrition security, increase crop and livestock production and reduce reliance on food imports, promote living standards and achieve a harmonious development of rural areas with a focus on skills development and job creation, within a context of improved management of natural resources and overall agricultural sustainability.

The sectors' indicators

The sectors' indicators by the end of 2016 showed that total value of the sectors' contribution to GDP raised from OmR (435.2) million in 2015 to OmR (506) million in 2016, with a growth rate (16.3%) which is the highest growth rate achieved over the last three five-year plans. The sectors achieved the highest growth rate between other economic sectors in 2016, in addition to that they contribute by approximately (35%) of the value of the food needs of Oman.

The total production volume was (1,844) thousand tons in 2016.The value of agricultural production about OmR (389) million in 2016. It covers one-third of Oman's food needs (almost two thirds of Oman's fresh food consumption).

Oman agricultural and fishery exports witnessed significant improvement where ttheir value increased from OmR 270 million in 2015 to OmR 294 million in 2016. Agricultural and fishery exports to total exports declined from 9% to 8.1% at the end of 2016, the proportion of export coverage of agricultural and fishery imports decline by a small percentage of (32%) in 2015, to about (30.9%) in 2016.

These indicators show a great potential for the growth of the agricultural and fisheries sectors as promising target sectors during the current five-year plan, it is expected to accommodate more jobs for Omanis, particularly in the context of the rapid growth of private investment supported by Governmental policy of promoting all economic sectors in the country. Agriculture and fisheries sectors, with the contribution qualify solid infrastructure, are expected to play a significant role in the economic development during the next period.

Agricultural development in Oman is faced with a number of dynamic challenges and constraints that have to be addressed properly in order to set agriculture into a sustainable progressive path. Natural resources limitation, including water scarcity and salinization, limited cultivable land, lack of skilled workers, in addition to inappropriate production practices, limited marketing and post-harvest infrastructures, and legal frame work and managerial efficiency.

Date Palm cultivation

Date palm sector in Oman is the first and largest agricultural activity in terms of cultivated area, more than (57428.65) feddans, it depends on the available water resources mainly ground water, aflaj, wells and freshwater springs. Studies on irrigation sources have shown that 47% of the agricultural holdings depend on wells, 38% depend on aflaj, and 15% of these holdings depend on more than one source of irrigation.

The number of date palms is more than (8.5) million, of which (7.5) million are in agricultural holdings, (800) thousand in house gardens and (200,000) palms are in streets and parks. Oman produced 355 thousand tons of dates (MoAF, Results of the Local Production Estimation of Dates, 2016).

Agricultural holdings

Agricultural holding is an economic unit with a single management. In Oman It includes agricultural production as vegetables, many types of suitable crops, fruit trees, date palms trees, field crops …etc. Table (1) shows the number of holdings, total area, and cultivated area according to the Agricultural Census of 2012-2013.

In 2013 there were 154000 agricultural holdings, covered about (355011) feddans. Al-Dakhliyah ranked first (31293) while Al-Westa had the lowest number of holdings. The cultivated area of that holdings about (170211) feddans, the biggest was at Al-Batinah N (59227), while Al-Westa had the lowest (398 feddan).

The estimated number of agricultural holding jump to about 190 000 in 2016.

Irrigation System

The total number of date palm holdings was (118275) covered an area of about (57429) feddans, of which only (7051) feddans irrigated with modern (controlled) irrigation systems, represent 12% of total, and (50377) feddans use the traditional system (aflaj and flood) system. Table (2). The governorates ranked in terms of controlled irrigation system started with Al-Batinah N (1580.5 feddan), and the lowest was Al-Westa (6.9 feddan).

Dates Production

In 2016 The number of productive date palms in Oman was (6,568,960) trees of different varieties, producing about (355) thousand tons. The overall average annual yield was (54.09) kg. Al-Batinah N ranked first in terms of the number of trees and production, Al-Batinah S was second and Al-Dakhliyah ranked third. Table (3) shows number of productive date palms (female only), average annual productivity, production of dates, and its percentage for 2016 season.

Top Productivity

In 2016 ARrustaq , ranked first in dates production, (36.2) thousand tons, (10.2%) of the total, and Bihla were the last willayats to produce more than ten thousand tons that year. Average tree's yield for all was (54) kg, ranged from about (131) kg/ tree in Al-Hamra to about (23) kg/ tree in Buraimi. (Table 5)

There is promising potential to double date production in Oman at least via working on reducing that variation between different locations and reach the optimal yield possible. Oman has the chance to do so.

Utilization of Domestic Dates Production

As shown in Table (6), date's domestic consumption is accounted for (185) thousand tons, with (60) and (20) kg per person consumed annually by Omanis and expatriates respectively. The quantity of dates consumed as animal fodder is (67) thousand tons while the export estimated at (15) thousand tons. Only (10) thousand tons of date processed. Current statistics showing that about (78) thousand tons (22% of total production) is suitable for processing, while the previous publications describe this percentage (about 20-24%) as "surplus" of table dates (suitable for human consumption) and "industrial date" (low quality or nonmarketable varieties). It might be as stock at the farm level, to be used by the farmers for their home consumption, gifts for relatives and neighbors, or as animal feed, or processing. *This issue needs more investigation, accurate classification and professional recording.*

First 10 Date Palm Cultivars

Oman has over than (300) varieties of dates, although the most popular are about (30). Most varieties are produced for human consumption, while some low quality, in test or low flesh to pit ratio..etc, used for feed or process. Naghal cultivar was the most produced in Oman, (35.14) thousand tons, about 10% of total dates production of year 2016. Table (7). *Most of these cultivars have been on top for the last decade. Maintain the specifications of the common varieties while working on improving them and introducing new one, according to local and export markets, is one of the important changes facing Omani date palm sector.*

Total production of the first (10) cultivars (246 tons of date) represent about (69%) of total dates production of 2016. As for the yield per tree, Mebsali recorded the highest yield (85.28 kg), Abo Daan (70.22 kg), Qhash Tabaq, not within top ten (69.02 kg), Madloki (68.53 kg), Khassab came fifth (66.56 kg) in year 2016.

Harvest, Post-Harvest, Processing and Marketing Activities

Dates are harvested and marketed at three stages, Khalal or Bisir, Rutab, Tamr although the most important stage is Rutab while the fruit is ideal for marketing as "dried" dates, they might have consumed year-round.

Post-harvest technologies, including all handling and storage operations, take place in the field and in processing plants. Field Sorting and Transporting, Processing includes fumigation, washing, storage, refrigeration, hydration, dehydration and curing. Grading of dates, is important to produce packed product uniform in size, shape, colour, and texture as good as possible and suitable for each variety, consumers and market requirements, food safety standards and requirements. As all agriculture products, dates proper storage and refrigeration maintain quality and fulfill current market demands, and ensure the added value benefits.

Omani dates marketing system is a simple one. The most important channels are dates processing units and factories, local markets and on-farm selling of dates, sometimes while the date still on the trees (locally known as Tana), and export.

Most Omani dates consumed locally, domestic markets are the most important as they absorb about 80% of the total annual production for the last 15 years. The main category of products is the dates, to be consumed fresh, hole date, processed products include pitted pressed dates, date paste, date syrup (called dibis). The other category by-products, such as the trunk, leaves, the male pollen, … etc.

Date Palm Processing

Through the current consumption of Omani dates, the low level of date industries of all kinds is evident. Based on the concept of food processing, which means the transformation / modification / conservation / manufacture of raw agricultural material to images that enables the user to circulate, save and consume easily in flexible times, and thus adds "additional value, financial or nutritional or physical to the basic/ raw material". The manufacture of dates and their products can play an influential role in their trade, whether locally or for export, especially if this is done with high quality standards and complies with the food safety and quality standards required by the markets.

In the field of industrialization, MoAF distributed machines and equipment for the processing and packing of dates as small units (production capacity of 10-20 tons per year). MOAF has allocated funds and efforts to support factories and existing date units to increase their production efficiency and increase their capacity.

Omani firms of date processing faced many challenges, which enable them to achieve their goals. A study to evaluate the status of those firms (*A Diagnostic Study to Evaluate the Status of Units and Plants of dates' manufacturing, done For MoAF, Directorate General for Planning and Development, funded by the Agricultural and Fisheries Development Fund 2013),* found that the number of factories (9) of which (2) were not working, (79) manufacturing units of which (45) were not working. The total design capacity in 2010 for working units was (2413 tons of date), total targeted capacity (1376 tons), while total actual capacity reached (736 tons). Thus, the percentage of actual exploitation (31%) and (45%) of the total design capacity.

The study also noted that the low rates of actual capacity utilization of design capacity, in most units ranged between (60% - about 6%). The owners of these units attributed the most important reasons ware lack of raw dates (the quantities required by market), or the lack of effective channels of marketing.

The study identified difficulties facing these firms, especially high fixed operating costs due to lack of access to optimum operational capacities, shortage of proper raw materials (date), that exacerbated the problem and led many of them to abandoned this vital activity.

Cost of Processing

The cost of the two services (manufacturing and packaging materials) was (50%) of the total cost, compared to the other half for the raw materials (dates and additives). Due to low design or operational utilization rates available to factories (most of them operate at less than (50%) of its designed capacity) and thus increased the cost of production per unit. Packaging materials and additives are often imported, which are subject to fluctuations in prices and flow in the local markets and availability to those who need it.

The MoAF has recognized the importance of food processing and has paid considerable attention to it over the past two decades, especially dates. The date palm development strategy included several programs and projects to maximize the economic, social and environmental returns from the date palm sector, as well as the capacity building of the human resources of farmers producing dates and those dealing with the sector in marketing, manufacturing, warehousing, research, extension and follow-up

Box No 1

In a report prepared by a group of experts and scientists, working on the Sustainable Agricultural and Rural Development Strategy 2040 (SARDS), for the (MoAF), covered cost-benefit analysis of policy options for the date palms value chain in Oman (2015), the report primary findings covered most related topics.

After calculating the production and income accounts, the margins (revenues, value added and profits) of the agents identified, the study show "that the small farmers producing high quality dates creates more value added that translates into higher profits. The small producers of medium and low quality dates obtain also positive profits from their production activities, although less than high quality producers".

Most date palm farmers usually cultivate other crops, fodder, vegetable, and occasionally citrus, …etc, the report noticed that these common practice of cultivation is profitable as “Small farmers also cultivated fodder in their holdings taking advantage of the space among the trees and the inputs used for the dates production”.

Also “the large farmers producing high quality dates creates more value added and therefore enjoy higher profits. The large producer of medium and low quality dates have also positive profits, although a bit and substantially less, respectively, than high quality producer, also in presence of a different irrigation technology. Despite modern irrigation, the large producers of low quality dates incur still in positive profits but they are lower than the profits obtained using traditional irrigation considering the extra costs to equip the holding with irrigation facilities not fully covered by the increasing revenues from higher yields.

“From the analysis of the upstream representative agents of the date palms value chain, it emerges that cultivating dates is in general a profitable agricultural activity for Omani farmers. Differences, however, emerges from the quality of fruits harvested: in particular, high and medium quality dates are more remunerative than low quality ones.

“Improved/modern irrigation is beneficial only for high and medium quality dates.

“For small and large farmers cultivating low quality dates, parts of the profits have to be attributed also to the fodder that is intercropped among the trees: without the revenues from these outputs, the date production activity would not have positive profits.”

Aggregating all the farmers to cover all the national production, the entire production and income account of the date palm value chain showing that almost 25% of the total revenues represent the profits and overall the date production can be considered a high value added agricultural activity, considering that the Net Value Added represents 60% of total output.

The date production is poorly mechanized considering the residual percentage of the fixed capital consumption, while the intermediate input cover almost the 37% of the total revenues. Among all the downstream agents in the date value chain, in Oman there are two representative categories, small processing units, and Large processing factories, both of them earn profits from their activity. The intermediate inputs from inside the chain (i.e. the raw dates) represent the major item in the total output value composition for the both the processors, representing more than 55 % of the total revenues. It is interesting underline that these inputs come from inside the chain because are bought from the upstream agents (i.e. producers, collectors and wholesalers) of the same date palms chain. Net value added represents most of the remaining part of the total output value accounting. The fixed capital consumption account for around 2.5% of the total output value and is composed mainly by the machineries used by for the processing activities.

All the segments of the value chain earn positive profits leading to the conclusion that no evident bottlenecks emerge from the accounting framework at market prices and there are no subsectors in the value chain that may harm the entire value chain being not profitable.

Feasibility studies for different processing factories of date products

There are clear evidences world-wide that agro- industries have a significant global impact on economic development and poverty reduction, in both urban and rural communities as such establishment of an agro-industrial project shall have significant effect specifically in the immediate vicinity of its location, and generally within the country as a whole.

The MoAF funded Technical and Economic Feasibility studies for the of different types of date products, in collaboration with well-informed consultant. Date Palm Processing Plant, Derived Date Project, Date Palm Feed Processing Plant, and Date Palm Wood Product Processing Plant.

The projects are expected to provide employment for the rural population in activities such as handling, packaging, processing, transporting and marketing. Through proper *implementation and utilization of available resources the projects will have a major positive impact on the standard of living of people in the area and participate in the GDP of the country with a sustainable annual growth rate.*

The first study, Date palm processing Plant (DPPP)

Al-Rustak factory was established in 1974 for packing local Omani dates and marketing its products locally and for export, due to number of management problems and production obstacles, the factory has stopped production and closed in the late nineties. The concept of (DPPP) is to establish a new modern date factory, on the same site of Al-Rustak factory or any suitable location, to utilize the available Omani dates for processing and marketing of high quality dates and dates products to the local and export market.

The main project's components are the plant and six collecting centers. The designed capacity is 30,000 tons/ year to be reached during three phases to produce (5) main product and (25) sub-products. The main products are Date Syrup (using Grade B dates), Chopped dates, Date Paste (using Grade B dates), Thermoform and pressed blocks, Loose date products, bulk and retail, pitted and non-pitted.

The overall processing/production procedure is in a systematic flow beginning with receiving, fumigation, sorting, washing, drying and packing.

The project concentrated on production of profitable products.

Late additional products can be added as per market requirements.

The second study; Derived Date Project (DDP)

The concept of the study is of date derived products through utilization of medium the production date varieties. Total annual amount of "surplus" or "available" date, as animal feed and post-harvest losses, is about (145,000) tons in 2016. Oman produces high quantities of "low grade" date varieties especially in the coastal areas, therefore, it is highly recommended to transform this category of dates, which are mainly soft, through valorization to valuable products by utilizing the high concentration of sugar in the date and applying physical, microbiological and chemical process of new and up to date technologies.

Major Products are Liquid Sugar (mixed glucose and fructose), Glucose, Fructose, Alcohols (Industrial and Medical), Organic acids (acetic, citric, oxalic, and formic acid), Yeast, and Vinegar. Other products are Liquid Glucose, Liquid Fructose, High Fructose Date syrup (HFDS), Seed Coffee, Seed Oil, Caramel, Carbon dioxide as by-product, and Fiber products (Flakes).

Market Study shows product screening process, where before selecting the derived products, compare the economic feasibility of the different date derived products in the local, regional, and international markets. The analysis identified the most promising products that should be focused on. It also focused on the products which were ranked the highest among all the other derived products.

Economic Impact: DDP is considered one of main agro-industrial projects in Oman. It will create value added products which have economic value in the food and pharmaceutical industry. The project will increase the date palm farmer's revenue as well as other sectors and activities. The end products as well as the by-products values are much higher than its original 2nd and 3r grade of dates which represents (60-70%) of the dates produced in the coastal areas. All the premiums of the infrastructure are considered as added value to the overall economic value and the GDP of the country. The project is expected to generate direct employment opportunities for Omanis.

Social and Environmental Impact: In addition to supporting the farmers (by ensuring better realization for products) their project shall also channelize part of the profits towards community programs that shall ensure the better education, health and improvement of the overall standard of living of the communities in remote villages where dates are grown.
The project will protect the environment through maintenance. The eastern coast of Oman is the most affected areas from high-temperature and moisture and to high level of ground water salinity. By removing the low and deteriorated dates from the farms, this will participate in saving that area from pollution.

DDP will use approximately (48,000) tons of low quality dates or varieties suitable for this industry. It is participating in the socio-economic development of the date farming community. It is going to be the first in Oman, as well as in the region, to use date palm for the production of various date derived products of these capacities. It will produce by-products of economical values, to be used in Oman, and will support the profitability of the project.
Although, DDP requires a relatively large initial investment, the actual operational viability is very good giving a 44% gross margin. This viability is very sensitive to the price of the products sold and that a relatively small change in the price can improve or decrease the viability of the project.
The feasibility study has been established based on conservative assumptions regarding the capex as well as opex. Some proposed changes are expected to enhance the profitability.

The third study; Date Palm Wood Processing Plant (WDPP)

This part summarizes the technical and financial feasibility study for the Date Palm Wood Processing Plant (WDPP) Project. The major raw material to be used for producing wood should come from date palm waste (leaves, trunks and other parts). However, it is suggested that wood waste and Mesquite can be used as a source of wood raw materials as well.
Farmers usually either sell the date palm waste (eaves/ saaff as bundles "daan") or burn it. An efficient date palm collecting schemes is required to assure the continuous supply of date palm leaves as the major raw material to the project. A suitable collection system is also required for collecting wood waste and Mesquite.

The Objectives of WDPP are production of products of economic use (wood) from agro-industrial waste, add to the farmers' income by purchasing date palm waste on regular basis, and reduce Mesquite population and its area.
For MDF, an advanced technology and processing is suggested to improve the quality of fiberboard, these include innovations in wood preparation, resin recipes, press technology, and panel sanding techniques. Advanced press technology will be used to shorten overall pressing cycles, while anti-static technology will contribute to increase belt life during the sanding process.

Considering the detailed assessment of potential products, the Market study concludes that focus on the domestic market as well as exports a MDF plant is recommended. Currently molded pallets are not used in Oman, the product has to compete in the market as a substitute for wooden pallets in terms of pricing. Plastic composite doors are currently not available in the market, hence aggressive marketing efforts need be carried to promote them, the superiority of the product in terms of price, quality, durability, finish, ...etc.

The fourth study: Date Palm Feed Processing Plant (DPFPP)

Oman has a good livestock population, in 2014 there were (3,300,500) head of livestock. Oman depend heavily on imported raw materials to produce concentrates and other supplementary feeds. local roughage production is limited to Rhodes-grass and Alfalfa under irrigation, some hay is imported as well.

There are several feed mills in the country, flour mills Co. SAOG, is the largest supplier. No feed plant in the region is using date palm by-products commercially as raw material.

The DPFPP objective is to establish a profitable animal feed plant producing ruminant feeds incorporating under-utilized or wasted date palm by-products.

The date palm leaves will be chopped and treated with urea and steam to improve the digestibility and convert it into a raw material with a reasonable nutritional quality, rich in crude fiber. Competition will mainly focus on the price of the feeds. Both the (DFPP) project and the (DWPP) project use the date palm leaves as one of their major ingredients. Therefore the Consultant proposes to establish these factories close to each other. The DFPP site is close to the port, has the needed infrastructures and in the middle of the date production region.

Considering the possibility of collecting about amount of (15 to 20) kg of leaves from each date palm, it is expected to get about (96 to 128) thousand Mton of raw material annually. The leaves to be collected by contractors, in some collection zones and using movable choppers to ensure efficiency.

Economic and Social Impacts of the Project

- Through treating and processing of the date palm leaves this under-utilized product is converted into a raw material to produce long fiber feeds.
- The proposed technology allows using law quality dates in the formulation similar to the farmers practice.
- As a specialized feed mill, it will contribute to development by increasing the output of the manufacturing sector and a profit generating business. This is in line with the Government strategy to use the date palm by-products.
- The produced long fiber feeds will make it possible for the livestock holders to reduce their dependency on irrigated roughages or imported.
- The long fiber feeds (Roughage Replacement feed and High Fiber feed) can reduce the consumption of roughages by 30-70%.
- The big social impact will be enhancing the traditional usage of date palm leaves and by-products and transfer them into a valuable feed resource.
- It will contribute to the sustainability of the date palm production in Oman.
- Provide jobs opportunities for some Omani workers to the maximum possible extent.

Development and Environmental Impacts

- The shortage of irrigation water and consequently roughages, can be partly overcome through the feeding of the long fiber feeds.
- Agricultural enterprises in Oman are embedded long history. The date palm by-products have always been used as much as possible but in modern society pose a waste burden. The Project will use these date by-products (date palm leaves, date processing by-products, date stones and date pulp).
- The grinded and treated date palm leaves will become a replacement for the imported roughages hay and straw.Low quality dates and date pulp could be used in this Project depending on the price.

AFDF as Date Palm Sector Supporter

Agricultural and Fisheries Development Fund of Oman(AFDF) financed (26) development projects, until 2016, targeting date palm sector, with a total cost of about OmR 5.2 million (about US$ 13.5 million). Twenty two projects are all components and their results have a direct developmental effect on Date palm, other projects of indirect effect. Since its inception in 2004, AFDF has financed (222) development projects targeting the agricultural and fisheries sectors in the Sultanate of Oman at a total cost of OmR 39 million.

AFDF was the second place winner, for best development project, of Khalifa International Date Palm Award 2011, the pilot prize and its renewable thinking and practices, in addition to other forms of positive economic and environmental impact on Omani agriculture and fisheries' sectors. **www.afdf.gov.om.**

CONCLUSION AND RECOMMENDATIONS

1 Date palm constitute an important and sustainable source of agricultural income for Oman, it is competitive under certain conditions.
2 Even though, Oman has high quality varieties, they are never branded nor promoted as they should.
3 There is promising potential to double date production in Oman at least via working on reducing that variation between different locations and reach the optimal yield possible.
4 Environmental issues need to be taken into consideration as well, such as the use of depleted treated irrigation water, the use GM varieties, tolerant to drought, heat, major diseases, pests, and others issues
5 Considering other production side issues, reaching best quality standards remains one of the key challenges affecting the date palm value chain in Oman.
6 Most of Omani top 10 cultivars have been on top for the last decade. Maintain the specifications of the common varieties while working on improving them and introducing new one, according to local and export markets, is one of the important changes facing Omani date palm sector.
7 Oman needs to raise processors, factories and units, capacity. Utilized processing capacity to the designed capacity or at least to the available capacity of current factories and processing units.
8 One of the most important weaknesses is low level of technology of processing units. Manual labour, mainly at the processing stages, is one of the main components of costs reduction and improve economic efficiency.
9 Raise the quality of the processed dates, and their products, and increase their competitiveness, according to the best food safety requirements and local and international standards.
10 Proper Manager and his qualified team, including Total Quality Management, of the date palm processing firm has to be present in the plan of the investor or decision maker.
11 The last not the least is the week marketing of Omani dates as raw in different shapes, Khalal/ Bisir, Rutab, or Tamr, or date and its products. Current markets are important but new markets is the real challenge.
12 Investment to raise Omani processors' capacity and their marketing functions is necessary to translate improvements from the production side to the market level, both for domestic and international trade opportunities.
13 The statistics of date usage and utilization needs more investigation, accurate classification and professional recording.

14 Introduction of innovative products, techniques, marketing, and managerial aspects to date palm sector should achieve the ultimate goals sustainable efficiency and productivity.
15 Oman Food Investment Holding Co. (OFIC) had started investment in date processing after proficient investigation of local and international markets.
16 There to are proper investment opportunities, qualified investors may take a chance to evaluate them, update any required information, to make a suitable success story. AFDF may help in such efforts.

REFERENCES

Agricultural Census 2012-2013. Ministry of Agriculture and Fisheries, Oman.

AFDF (2016), www.afdf.gov.om. Agricultural and Fisheries Development Fund of Oman.

MoAF (2017), Reports about Technical and Economic Feasibility studies for Date Palm Processing Plant, Derived Date Project, Feed Processing Plant, and Wood Products Plant. Ministry of Agriculture and Fisheries Wealth, Oman.

MoAF (2016), Annual report. Ministry of Agriculture and Fisheries Wealth, Oman.

MoAF (2014- 2016), Statistical Yearbook. Ministry of Agriculture and Fisheries Wealth, Oman.

MoAF (2014), A Diagnostic Study to Evaluate the Status of Units and Plants of dates' manufacturing, MoAF, Directorate General for Planning and Development, Oman.

MoAF (2014), Cost-Benefit Analysis of policy options for the date palms value chain in Oman (unpublished report). Ministry of Agriculture and Fisheries Wealth, Muscat, Oman.

hhp://www.omanfood.com.om. Oman Food Investment Holding Co. (OFIC) site.

http://www.maf.gov.om. Ministry of Agriculture and Fisheries site.

Tables

Table 1: Number of Agricultural Holdings by Governorate, Cultivated Area, 2013

Sq	Governorate	No of holdings	Cultivated Area (fed)	Total area (fed)
1	Al-Dakhliyah	31293	17969.80	45732.97
2	ASharqiyah N	24284	13539.80	27523.27
3	Al-Batinah N	21777	59227.14	85118.27
4	Al-Batinah S	19532	25805.34	48984.53
5	Al-Dhahirah	15406	11817.45	33295.08
6	ASharqiyah S	12861	7810.39	15206.87
7	Dhufar	11498	17778.89	65921.13
8	Muscat	8345	6194.43	11555.85
9	Al-Buraimi	4504	7508.58	16123.21
10	Musandam	3871	2170.27	3242.0
11	Al-Westa	630	389.12	2307.90
	Total	**154010**	**170211.27**	**355011.12**

Source: Agricultural Census 2012-2013 (Ministry of Agriculture and Fisheries)

Table 2: Number of date palm holdings, area (feddan), and irrigation system, 2013

Governorates	No of DP Holdings	Cultivated Area (feddan)		
		Controlled Irrigation	Traditional Irrigation	Total
Muscat	6772	488.43	2902.22	3390.65
Dhufar	876	953.72	415.42	1369.15
Musandam	2758	27.23	1469.67	1496.90
Al-Buraimi	4180	666.47	2815.54	3482.01
Al-Dakhliyah	28035	951.90	8532.85	9484.76
Al-Batinah N	17363	1580.51	10869.13	12476.75
Al-Batinah S	17666	1395.10	6414.13	7809.24
ASharqiyah S	6936	197.74	4020.03	4217.77
ASharqiyah N	19737	321.53	6924.25	7245.79
Al-Dhahirah	13766	461.88	5671.96	6133.85
Al-Westa	76	6.94	314.89	321.83
Total	**118275**	**7051.50**	**50377.14**	**57428.65**

Source: Agricultural Census 2012-2013 (Ministry of Agriculture and Fisheries)

The situation had improved relatively during the last five years, but a lot of work is required to improve water use efficiency, date palm productivity, sustainability and profitability.

Table 3: No of Date palm trees, annual yield (kg), and date production, 2016

Sq	Governorate	trees contribute to production(000)	Average tree's yield (KG)	Production (ton)	Production %
1	Muscat	346,250	37.02	12,817	3.61
2	Musandam	232,629	30.20	7,024	1.98
3	Al-Buraimi	456,997	24.21	11,063	3.11
4	Al-Dakhliyah	1,026,694	63.60	65,297	18.38
5	Al-Batinah N	1,528,274	48.93	74,779	21.04
6	Al-Batinah S	929,948	75.12	69,861	19.66
7	ASharqiyah S	464,316	78.51	36,454	10.26
8	ASharqiyah N	826,948	46.59	38,531	10.84
9	Al-Dhahirah	691,900	51.27	35,472	9.98
10	Dhufar	65,006	62.04	4,033	1.13
	Total	**6,568,960**	**54.09**	**355,332**	**100.00**

Source: MoAF- Directorate General for Planning and Development, 2016.

Table (4) shows date production for the last five years. Al-Batinah, North and South, and Al-Dakhliyah are the main production areas.

Table 4: Yearly Dates Production by Governorates, 2012-2016 (in thousand)

sq	Governorate	Production of Date (000 tons) for years				
		2012	**2013**	**2014**	**2015**	**2016**
1	Muscat	10.8	10.1	10.4	12.6	12.8
2	Musandam	6.7	6.5	8.3	6.9	7.0
3	Al- Buraimi	6.6	13.1	16.2	10.5	11.1
4	Al-Dakhliyah	52.0	63.2	66.7	61.0	65.3
5	Al-Batinah North	68.9	86.4	60.1	73.5	74.8
6	Al-Batinah South	55.0	49.7	62.6	68.7	69.9
7	ASharqiyah South	24.2	15.0	30.8	35.3	36.5
8	ASharqiyah North	28.8	29.4	27.1	37.5	38.5
9	Al-Dhahirah	28.3	34.9	34.0	34.7	35.5
10	Dhufar	-	-	-	4.0	4.0
	Total	**281.3**	**308.4**	**316.1**	**344.7**	**355.3**
	Index No (2012)	**100**	**110**	**112**	**123**	**126**

Source: MoAF- Directorate General for Planning and Development, 2016.

Table (5): The Most Productive Willayats during season 2016 (000 tons of date)

sq	Willaya	Production (Ton)	% from Total production	Average tree's yield (Kg)	No of date palms*
1	ARrustaq	36,241	10.20	109.69	330,384
2	Suhar	32,008	9.01	76.87	416,370
3	Abri	23,755	6.69	51.78	458,719
4	Asswaqe	17,464	4.91	48.06	363,387
5	Mudhabi	16,579	4.67	49.43	335,399
6	Barka	14,875	4.19	60.98	243,949
7	Nizwa	14,078	3.96	94.32	149,264
8	Samayel	12,166	3.42	53.32	228,171
9	Jaalan bani BuHasan	11,975	3.37	74.62	160,476
10	Saham	10,640	2.99	34.71	306,552
11	ElKamil Walwafi	10,439	2.94	82.62	126,351
12	Bihla	10,204	2.87	56.94	179,192
13	Khaboura	9,373	2.64	37.84	247,725
14	Izki	8,229	2.32	52.80	155,842
15	Sur	7,891	2.22	87.23	90,456
16	Musanaa	7,739	2.18	45.32	170,757
17	Qurayyat	7,178	2.02	36.78	195,181
18	Manah	6,911	1.94	102.07	67,709
19	Elhamra	6,112	1.72	113.17	54,009
20	Jalan Bani Bu Ali	6,080	1.71	70.56	86,161
21	Yanqul	5,955	1.68	58.67	101,497
22	Bidiyah	5,826	1.64	56.42	103,255
23	Dhanak	5,762	1.62	43.76	131,684
24	Nakhal	5,337	1.50	61.78	86,384
25	Elkabil	5,199	1.46	53.31	97,524
26	Demaa Waltaien	4,973	1.40	30.17	164,819
27	Mahadha	4,554	1.28	25.62	177,782
28	Thamrit	4,033	1.13	62.04	65,006
29	Adam	4,012	1.13	41.73	96,157
30	Bidbid	3,584	1.01	37.20	96,350
31	Buraimi	3,389	0.95	22.83	148,437
32	Other	32,769	9.22	35.08	934,011
	Total	**355,332**	**100.00**	**54.09**	**6,568,960**

*Female tree contributed to the production. Source: MoAF Statistics, 2016

Table 6: Utilization of Domestic Dates Production (thousand tons), 2013

Utilization	000 ton of date
Human consumption	185
Exports	15
Processed (industrial)	10
Suitable for processing	78
Animal fodder	67
Total production of date	355

Source: MoAF- Directorate General for Planning and Development,Date palm Statistics, 2016

Table 7: First 10 Date Palm Cultivars , 2016

sq	Cultivar	Production 000 ton	% of Total Production	Yield (kg/Tree)
1	Naghal	35,145	9.89	54.61
2	Khassab	33,858	9.5	66.56
3	Khlass	33,452	9.4	56.29
4	Mebsali	31,431	8.8	85.28
5	Um Silla	29,366	8.3	50.06
6	Fardh	23,455	6.6	49.23
7	Khnaizi	20,266	5.7	46.83
8	Shahel	19,180	5.4	54.02
9	Abo Daan	10,113	2.8	70.22
10	Madloki	9,799	2.8	68.53
	Total	**246,065**		

Source: MoAF- Directorate General for Planning and Development,Date palm Statistics, 2016

Table (8) Date processing units and factories products (2008-2010)

Product Type	Processed Dates Production (Tons)			% of Increase (Decrease)/Base Year	
	2008	**2009**	**2010**	**2009**	**2010**
Packed Date	137.0	116.75	92.2	(10.1) -	- (33)
Date Paste/ Madlook	45.1	438.10	573.4	(-) 3.0	27.4
Total	587.6	54.85	665.6	(-) 5.6	13.3

Source: MoAF- Directorate General for Planning and Development, 2013

The trajectory of evolution of the date palm chain in the Ziban region (Algeria), situation and prospects."

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Abstract

The region of Biskra is one of the most important phoenicultural regions in Algeria in production (quantity and quality). Like the other phoenicultural regions of the country, the study area has benefited a lot from deferent agricultural development programs since 2000 to date. Through this intervention, we will try to highlight the reality of this date chain in the Ziban region and analyze its dynamics of evolution through these programs. And from a SWOT analysis, present these assets, opportunities, threats and weaknesses. The results obtained illustrate that the impacts of State actions are very significant in this region, and that the results of the main indicators analyzed are very fruitful and improve from year to year; although the goals are not reaching fully; because of the combination of a series of technical, socio-economic, natural, agricultural and administrative constraints. On the other hand, the SWOT analysis indicates that this dynamic evolution is undoubtedly due to the presence of several assets of this sector and by the opportunities offered in this region and at national and international level. But also by the effectiveness of the strategies applied to face the different threats and constraints that hinder this sector at all levels that are also numerous.

Keyword: the dates sector, technical-economic indicators, dynamics, SWOT analysis, Ziban, Algeria.

1. INTRODUCTION

In the Saharan regions, agriculture has always been a key element in development. On the one hand, it constitutes on the economic level a significant activity for the oasis populations (Bouammar, 2010). Through the deferential agricultural policies and agricultural development programs led by the Algerian state, this agriculture has gone through several stages and the results obtained are very appreciable at present.

The date palm is the keystone of the oasis ecologically and economically and represents an important link in the mitigation of an austere climate and fulfills undeniable ecological social and economic functions that should be made explicit (Bouammar, 2010). Date palm cultivation occupies an important place in the oasis agricultural production system in Algeria and constitutes a major resource for the majority of the inhabitants of the Saharan regions of the country (Nourani, 2016).

Usually named the capital of Ziban, the wilaya of Biskra is one of the most important phoenicicole regions in Algeria, in terms of the number of palm trees and production; also at the quality scale; following the famous variety Deglet Nour (Benziouche 2016). But also, from the point of view of biodiversity within the oasis, it has about (300) different cultivars (Boughlita 2017.(Like the other phoenicicole regions of the country, the study area has benefited a lot from deferent agricultural development programs since 2000 to date. Several studies have shown that the impact of these agricultural programs varies from one sector to another and from one region of the country to another.

Through this intervention, we will try ***to highlight the reality of this date chain in the Ziban region and analyze its dynamics of evolution through these programs***. Our objective is also ***the evaluation of the agricultural policies carried out by the government for this culture***. This is based on an analysis of the main technical and economic indicators upstream and downstream (area, phytosanitary heritage, production, yield and exports and also conditioning and recovery). And from a SWOT analysis, present these assets, opportunities, threats and weaknesses.

2. MATERIALS AND METHODS

Our methodological approach to realize this work combines two phases and complementary tools; a bibliographical study was conducted on the subject. This made it possible to specify the problematic of the study and to collect the data relating to this date die and this region of study. In the second place, several contacts with resource persons having a relationship with the subject of study were held in order to identify certain data necessary for carrying out the investigations. In the third step, we used all our experiences of our field surveys in the palm groves of the region, also the results of our scientific research on this research topic. Finally we also based on studies and statistics from the Ministry of Agriculture and Agricultural Services of Biskra.

A series of analysis of the technical and economic indicators of the sector, like the evolution dynamics of the date palms area, the number of palm trees, the production, the yield and exports and also conditioning and valorization. In this context, several figures of several synthetic forms are drawn up. The combination of these two tools made it possible, among other things, to proceed from a SWOT analysis, to a presentation of the Strengths, Opportunities, Threats and Weaknesses of phoeniciculture in the study area. Completion of the work will be done by proposing the necessary recommendations to deal with the constraints and threats and to exploit the opportunities that exist in the political, sociological, economic and technical environment with the essential aim of promoting the phoenicultural activity in the wilaya of Biskra.

Context of the study area

Biskra is a phoenicultural site, extending over an area of 216,671.2 km2 and 425 km south-east of the capital, located between 4 ° 15 'and 6 ° 45' East longitude and between 35 ° 15 ' and 33 ° 30 'degree North latitude (Benziouche 2016). According to Aidaoui (1994), the relief of Biskra consists of 4 morphological units. It is the wilaya most rich in water resources. The aquifers represent the essential hydraulic capital of the wilaya (Aouidane, 2008). Irrigation is based exclusively on groundwater and the operation of the two existing dams. The region is known for very high temperatures (Max 42 C °) and low rainfall (an annual average of no more than 139 mm). The rains fall irregularly and can be torrent. As a result, the dry phase is extended and sometimes spreads throughout the year (Benziouche 2016).

3. RESULTS AND DISCUSSION

3.1.Technical and economic performance of the Ziban date chain.

The Biskra region occupies a very important place in the Algerian phoenicultural map from the point of view of palm number and production (quantity and quality). Phoeniculture covered almost 41% of the UAA of the wilaya (figure 2.) which represents 24% of the national phoenicultural area; it also occupies the first place, with more than 25% of the date palm at the national level (figure 3).

3.2. Evolution of the number of date palm in Biskra

According to the statistics of the DSA, 2016, the date palm occupies an area of more than 43105 ha which represents 23% of the UAA of the wilaya. This area contains 4.31 million palms, of which 3.98 million productive palms or 92.34% are productive. The wilaya of Biskra shows a very slow evolution of the number of palm trees since the eighties. Between 1984 and 1999, about 658,300 palms were planted (Bouammar, 2009), an average of 43,000 palms / year. In the year 2000, the number of palm trees was 2,460,170 palms, in 2007 this potential saw a clear increase of 4,127,800 palms, that is an increase of about one and a half million palms in the space of 7 years, and currently the palm number is made up of 4.31 million palms, (Figure 4).

The palm grove of Biskra has had a very interesting annual evolution in terms of the number of productive palms thanks to the development of large areas after the promulgation of the law of the APFA (Access to Agricultural Land Ownership) and the development programs. the GCA (General Agricultural Concessions) and the PNDA (National Agricultural Development Program) (Benziouche and Cheriet, 2012) (Benziouche, 2016). In addition to the motivation of the local population and its know-how. (Ben Sayah, 2014.(

However, the analysis shows a dispersion of this phoenicultural potential on the different municipalities of the wilaya. Indeed, Sidi Okba and Tolga present the largest number of palm trees with 15% and 12% respectively of the total of date palms of the wilaya followed by Lioua with 10%, Oumache and M'Lili with 9% for both. The contribution of the other communes and well illustrated in Figure 5. The structural analysis of this date palm illustrates that the lion's share is captured by the cultivar Deglet Nour with 62%. Second, the 25% dry and assimilated cultivars are followed by the Ghars and similar varieties (13%) (Benziouche 2016) and (DSA Biskra, 2016) (Figure 6).

The Deglet Nour with high market value prevails in the western municipalities of the wilaya with 80.24% (Luchana, 86% Leghrous, 83.5% Bordj ben azouz), and to a less degree in the municipalities of the East of the wilaya with 60 % of the total palms of these regions in 2016. The importance of this cultivar does not exceed 58% and 53% in the communes of the center and the south of the wilaya respectively. However, the other categories of cultivars are dominated in the communes of the East and the South and center of the wilaya. This polarization is due mainly to the adaptation and the specificities and requirements of each cultivar to the soil and climat conditions of each region. Particularly for Deglet Nour with a limited geographical area, very demanding and very sensitive to diseases.

Closely correlated with the situation and the number of palms in production as well as with the biological alternation of palms (Benziouche and Chehat, 2010), the cultural management and the climatic hazards (Benziouche and Cheriet, 2012), the production of dates of Biskra is variable according to the campaigns. This production has experienced a great growth, it is increasing from one year to another, passing from 60000 T in 1990 to 370 000 T in 2015; an increase of 310%, and it is close to 407788 1T in 2016 (Figure 8).

As with the number of palm trees, the production of Biskra dates is mainly concentrated in the Zab Gharbi region (municipalities in the west of the wilaya), mainly in the Tolga, Ourelal and Foughala dairas, which account for 55% of the production. of the region. The municipality of Tolga monopolizes the most important production. It represents 10.44% of the total production, ie 425820 Qx, followed by SidiOkba with 8% and Leghrous with 6%. The share of other communes is illustrated in Figures 11 and 11.The latter are specialized especially in market gardening and other cultivars in addition to Deglet Nour (Abdelaoui 2016).

From the varietal point of view, the Deglet Nour variety covers most of the date production for the majority of wilaya communes except for Ourelal and M'lili where the Mech-Degla variety and its analogues dominate the other two varieties (Deglet Nour and its analogues and Ghars and analogues). Figure 12 describes in detail the date production of the three varieties and its analogues for each commune.

The lion's share of this production is Deglet Nour (65%) (Figure 9). More than 89% of this variation in production can be explained by the entry into production of new plantations, but also by the importance given to this sector by the State (Benziouche, 2012, Benziouche, 2016, Benziouche, 2017). It can also be explained by efforts to expand plantations, (Abdelaoui 2016). At the national level the wilaya of Biskra occupies the first place by 41% of the national production in 2016.

In the 2016 farming season (Figure 9), out of a total production of the wilaya of 407 thousand T, the Deglet Nour takes 265 thousand T (65%), followed by dates called "dry dates" (Mech Degla and other) with 22.38% this "soft dates" (Ghars) which total 51.1 T (or 12.6% of the production of the wilaya during this companion) (DSA Biskra, 2016.(

This polarization in quantity and quality of date production in the study area is undoubtedly due to the variation of the soil conditions (soil type, structure, fertility) and the quality of water availability and quality (pH conductivity) (Khechai et al, 2013) and irrigation techniques 5 (modern or traditional). Added to this is the mastery of the technical itinerary specific to these crops and the availability of local know-how for each municipality, but especially the deference to the importance of the market value of each cultivar on the markets.

3.3. Evolution of yield of dates palms of Biskra

Although this production is on an upward trend, it remains relatively weak and stagnant compared to the phoenicultural potentials put in place and the objectives drawn, as well as in relation to the production of the competing countries but also with regard to the expected results and objectives.

Varied from one year to another, from one variety to another and even from one commune to another; the average productivity per date palm at the level of the wilaya of Biskra is 72kg / foot in relation, during the period 1990-2016, all varieties combined. Its maximum is recorded in 2016 with 85 kg / foot and the minimum in 1990 with 54.94 kg / foot (Figure 13.(

These average yields, although they are higher than the national average, remain low and below the expected standards of 100 kg / foot and yields recorded in the US or Tunisia are 100 and 80 kg / foot respectively. (Benahmed, 994).

The analysis by municipality shows that the best average yields in 2016 per foot are recorded in the oases of Elghrous, Luchana, Tolga and Bordj Ben Azouz 160 kg / foot 146, 144, 139 and 131 respectively all varieties. While in the eastern communes of the wilaya does not exceed 60.kg/foot with a minimum of 50kg / foot el kantara. However, it should be noted that while some growers produce up to 160kg / ft in Tolga and Elghrous for the Deglet Nour variety, others do not exceed 40kg / ft for the same variety.

The highest yields per foot are recorded by the variety Deglet Nour with an average of 90kg / palm. The other varieties, Mech Degla and Ghars do not exceed 60 and 55 kg / palm respectively. Overall, the results show that there is a general trend towards increasing the yield of date palms in the study area during the analysis period.

Although national yields of date palms do not exceed 52 kg / palm in 2016; Nevertheless, the best average yields per foot are recorded in the oasis of Biskra. The edaphic and pedoclimatic specificities, as well as the cultural management and the value of the varieties; explain the importance of production and yields in the region and the positive impacts of state policies in this sector.

3.4.An industrial packaging fabric of dates in development

Before the 2000s the wilaya has 5 conditioning units with different capacities, and following the economic reforms, several private units of date conditioning, of different capacities and technical levels, were realized. In other words, it is since 2000 that the industrial fabric has undergone a profound change, within the framework of the National Plan for Agricultural Development (NADP), marked by the improvement of the conditions of transport, storage and packaging of dates, but also by promoting a new packaging activity in accordance with the requirements of international standards. The program encouraged the maintenance and modernization of old packaging units, as well as the increase of storage capacity and the creation of new units (Benziouche, 2012). In this context, some new packaging companies have established themselves or have extended their activities to the date sector. In addition to larger companies, there are a large number of small (artisanal and semi-industrial) units that sort and package for the local market. They buy prefabricated boxes with the name Deglet Nour (Benziouche, 2014. Other units are in the pipeline and many investment cases in the area of date packaging have been filed at the banks level, and at the level of the agricultural service directorates (DSA), and are waiting for the final agreement.

Since this restructuring, the conditioning of dates has also made an industrial fabric of about 29 units with different capacities; more than 50% of the total packing units at the national level.

3.5. SWOT analysis

3.5.1. Strengths

- Willingness and commitment of public authorities for the promotion of phoeniciculture
- Availability of diversified natural potential of the wilaya: edapho-climatic conditions favorable to phoeniciculture,
- •resence of trained managers in Saharan agriculture, who can in turn transmit the acquired knowledge,
- The existence of modern palm groves (no aging of palms and producers)
- Level of cultivation in the palm groves of the region of study very accepted.
- The dominance of Deglet Nour with high market value in domestic and export markets.

3.5.2. Weaknesses

- The advanced age of the plantations in certain communes particularly of the east of the wilaya.
- In some Oases of the Region, the phytosanitary situation is not good and very disturbing.
- The high price and the lack of good quality packaging on the market, and the problem of transporting crops;
- Irrigation in some oases in the study area is poor and worsening, especially with the increase in area and the low or even stagnant mobilization of water resources in this region
- The traditional irrigation system that requires rehabilitation, unequal exploitation of existing water between palm groves, the absence of irrigation techniques . .

- Poor mineral fertilization due to the shortage of chemical fertilizers. Quantities available in the markets are low and expensive for farmers.
- Producers' difficulties in financing their activities .
- Insufficient agricultural extension at the farm level; which diminishes the quality of the producers' know-how.

3.5.3. Opportunities

- Availability of very important water resources untapped
- Soil and climate conditions favorable for quality production
- Availability of local know-how and know-how very important in the region
- Possibility of financing and state support
- The existence of a buoyant and solvent market
- The availability of Tolga Deglet Nour labeling as a local product will encourage exports of dates from this region.

3.5.4. Threat

- Reduction of the water table and the appearance of diseases and pests.
- The development and competition of greenhouse and other speculations in the region
- Significant delay for exports and strong competition from neighboring countries
- Deficit organization of the profession in the region and the lack of coordination and synergies between the actors
- Absence of a standard and national standards for phoeniciculture.
- Insufficient awareness campaigns on agriculture
- The harvest of dates in the region studied is purely traditional, no mechanization is reported in the palm groves .
- The water deficit causes the deterioration of yields in quantity and quality and even the death of a large number of palm trees .
- The rise of the water table and the salinity of water and soil threatens the palm groves in some oasis.

SWOT analysis

Strengths

•Willingness and commitment of public authorities for the promotion of phoeniciculture

•Availability of diversified natural potential of the wilaya: edapho-climatic conditions favorable to phoeniciculture,

•Presence of trained managers in Saharan agriculture, who can in turn transmit the acquired knowledge,

•The existence of modern palm groves (no aging of palms and producers)

•Level of cultivation in the palm groves of the region of study very accepted .

•The dominance of Deglet Nour with high market value in domestic and export markets .

Weaknesses

• The advanced age of the plantations in certain communes .

• In some Oases of the Region, the phytosanitary situation is not good and very disturbing.

• The high price and the lack of good quality packaging on the market, and the problem of transporting crops;

•Irrigation in some oases in the study area is poor and worsening, especially with the increase in area and the low or even stagnant mobilization of water .•the traditional irrigation system that requires rehabilitation, unequal exploitation of existing water between palm groves, the absence of irrigation techniques . .

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•The water deficit causes the deterioration of yields in quantity and quality and even the death of a large number of palm trees .

•The rise of the water table and the salinity of water and soil threatens the palm groves in some oasis,

CONCLUSION

In conclusion, the main lesson we can draw from this work is that the dates sector in the Ziban region (Biskra) has experienced a boom and a dynamic of evolution very appreciable in all segments and levels of the date palm value chain, in upstream and downstream. As a result, major improvements have been recorded in all indicators and technical and economic variables calculated (production, area, number of date palm, yield, packaging and marketing ...). An improvement has also been recorded in the economic performance of the fish farms (evolution of income, profit and profitability) to this is added to the great social dynamics in the region thanks to the commercial activities related to this crop.

This situation is unquestionably explained by: first; the will of the public authorities to promote this sector like all the agricultural sector; through the implementation of several agricultural development programs. Second, the will of the actors, particularly the producers, to benefit from these programs. As a result, heavy investments have been put in place (planting palms, making fodder, etc.) and improving farming practices by the improving agricultural training. On the other hand, the sector remains very efficient from a socio-economic and environmental point of view as well.

Despite this dynamic and although the wilaya is ranked number 1 nationwide compared to the other 17 wilayas phoenicicoles Algerian, Nevertheless this sector is far, at all levels, conventional standards of this activity. These results remain insufficient and below expectations; and in relation to the actions undertaken during the last decade. This situation results from the combination of a series of constraints of a technical, socio-economic, natural, agronomic and administrative nature which hinder the development of this culture, notably that the world date economy has experienced a great evolution and the global distribution map of its economic indicators is undergoing much change.

All indicators remain poorly performing well despite the numerous and encouraging opportunities that are found in the political, economic, technical and social environment (PEST) of this sector of date palms in the study area. These opportunities should be exploited appropriately and in a timely manner at all levels, in order to develop and promote this value chain in the region. This will not be successful without: resolving constraints, coping and adapting with threats. But also by the application of the suggestions of the specialists.

REFERENCES

Aouidane L, 2008. Etude de la dynamique agricole dans la région des Ziban: cas de la zone d'El Ghrous. *Mémoire Magister, Univ Ouargla, Algérie, 205 p*

Benahmed G, 1998 Analyse de la filière datte en Tunisie. Mémoire de DEA ; ENSA de Montpellier France, 200p

Ben sayah F, 2014. Influence des conditions de stockage au froid des dattes sur leur qualité organoleptique dans la région des Zibans (Cas des dattes -variété Deglet Nour). Mémoire

Benziouche S.E., 2017. L'agriculture biologique, un outil de développement de la filière dattes dans la région des Ziban en Algérie. *Revue Cah. Agric. Vol 26, N°3.*

Benziouche S E., 2016. Les dattes biologiques comme outil de développement de la filière dattes dans la région des Ziban, Algérie. Proceding séminaire International, Gestion intégrée et durable des territoires oasiens, Tome 1, Maroc, pp152-264.

Benziouche S.E, 2014. Le conditionnement des dattes en Algérie Constats et perspectives » revue Roa Iktissadia, N° 4 ; Univ El-Oued, 2014.http://www.univ-eloued.dz/roa

Benziouche, S E 2013. Overview of economic analysis of production, consumption and export of dates in the region: current status, opportunities and challenges" Regional

Workshop on the Improvement of the Dates Value Chain in the Near East and North Africa Region Kuwait.
Benziouche S.E, *2012*. Analyse de la filière dattes en Algérie; constats et perspectives de développement. Cas de la daïra de Tolga. *Thèse de Doctorat ès agronomie. Alger: ENSA, 470 p*
Benziouche S.E, Cheriet F, *2012*. Structures et contraintes de la filière dattes en Algérie. Revue New Medit 4*: 49-57*
Benziouche S E, Chehat F, 2010. La conduite du palmier dattier dans les palmeraies des Ziban; (Algérie) Quelques éléments d'analyse. *Revue EJSR* 42: 644-660
Bouamar B, 2009 le développement agricole dans les régions sahariennes; étude de cas de la région de Ouargla et de la région de Biskra. Thèse de doctorat en science économiques Univ de Ouargla- Algérie.
Bouguedoura N, Bennaceur M, Babahani S, Benziouche S.E, 2015. Date Palm Status and Perspective in Algeria. Chapter in, Date palm Genetic Resources, Cultivar Assessment, Cultivation Practices and Novel Products. Springer: 125-168.
DSA, 2016 Direction des services agricoles de Biskra, rapport d'activité 2008. pp 2-14
Nourani, 2016. Etude conceptuelle des machines et proposition des techniques pour différentes opérations culturales en phœniciculture. Thèse Doc ENSA Alger, 122 p
Khechai S., Daoud Y., Boukehil K. 2013. Minéralogie et géochimie des sols des oasis des Ziban. *Séminaire national sur l'agriculture saharienne. Université de Biskra.*

Table

Table 1 : Structure of date palm cultivars by region of wilaya Biskra

Commune	Deglet Nour	GHARS	Mech Degla et autres
central of the wilaya	58,30	12,95	28,75
East of the wilaya	59,40	20,08	20,52
West of the wilaya	80,24	3,63	16,13
South of the wilaya	53,22	13,98	32,81

Figures

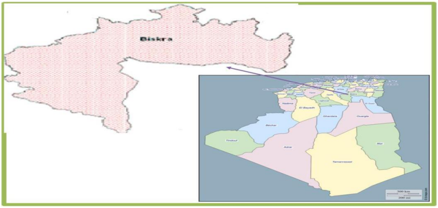
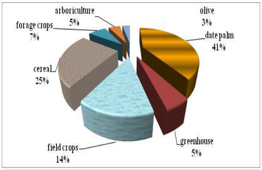


Figure 1 : Map of localization of the Ziban region (Benziouche 2017)



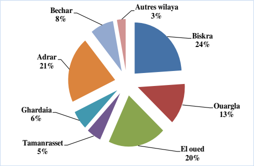


Figure 2: distribution of agricultural area by crops in the wilaya of Biskra

Figure°3 : Distribution of date palms of Algeria by wilaya in ***2016***

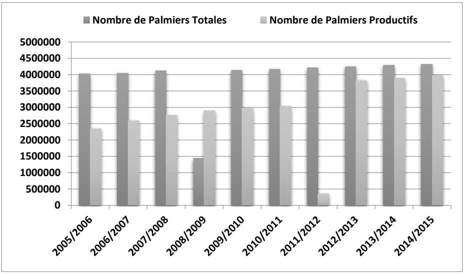


Figure 4 : Evolution of the date palm and date palm number in production of Biskra

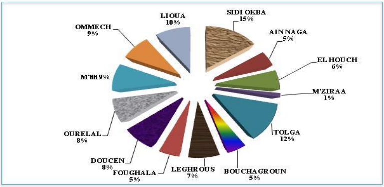


Figure 5: Distribution of date palms of Biskra by municipalities en 2016**.**

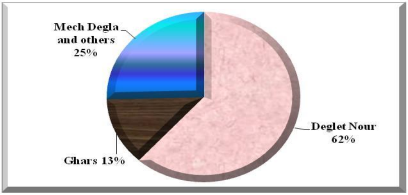


Figure 6 : Distribution of date palms of the wilaya of Biskra by cultivars in 2016

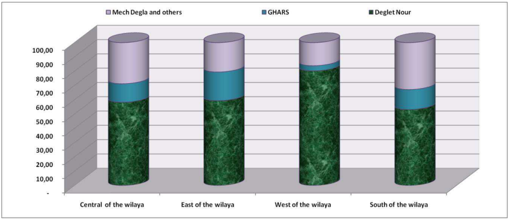


Figure 7 : Structure of date palm cultivars by region of wilaya Biskra

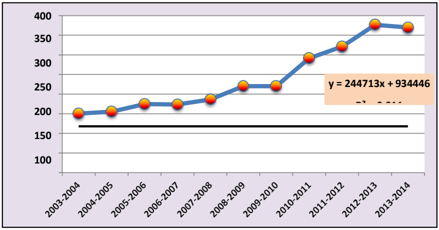


Figure 8 : Evolution of the production of the dates of the wilaya of Biskra (Abdelaoui 2016)

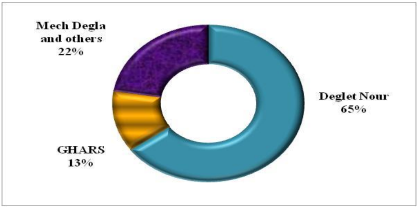
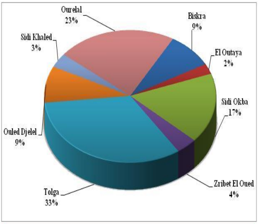


Figure 9: Structure of date production by cultivars in Biskra in 2016



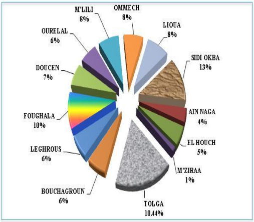


Figure 10 : Breakdown of dates palm production of Biskra by municipality in 2016

Figure 11 : Distribution of production dates by Biskra daïra in 2016

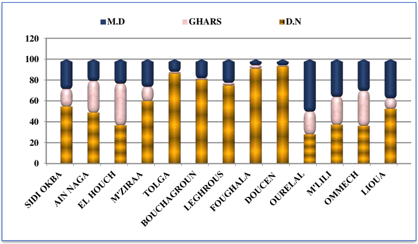


Figure 12: Structure of date production by municipality and variety in 2016

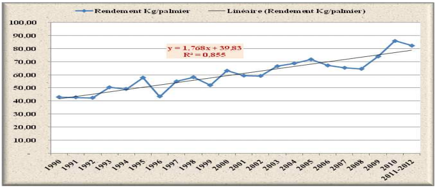


Figure 13: Evolution of date palm yield in Biskra .

Investigation of new cultivars of date palm (*Phoenix dactylifera* L.) raised from seed (pit) germination

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Abstract

Propagation of date palm, *Phoenix dactylifera* L. by seed is an easiest, cheapest and fastest method of date propagation, as compared to the propagation, by offshoots or through tissue culture. Date palms produced by this method are not true - to - type, and a wide range of variations is presumably expected in progeny. The present work represents a large scale breading experiment in which several thousands of date seeds (pits) have been planted in two separate locations at Al Ain city – Abu Dhabi, UAE and the seedlings produced were subjected to a continued observation from earlier stages of development until maturation and formation of spedices, were determining the gender becomes possible. Percentages of male and female palms were recorded, and other morphological features of vegetative and reproductive parts have been determined to help us recognize elite cultivars. In spite of the fact that propagation of date palm by seed commonly produces palms of low quality, yet the possibility of existence of some new cultivars that are of interest from the agricultural, nutritional and possibly of medicinal importance, should not be ruled out.

Among the interesting new cultivars selected was a cultivar that produces seedless fruit. Of the many other cultivars selected was cultivars that produce seeded fruit of good quality according to the known industrial, commercial and agricultural measures. Some cultivars resembling Barhi, Khlas, Maghool, Sultanah and other elite cultivars were among those that have been selected. These will be subjected to further studies; including fruit quality characteristics, physico-chemical properties in addition to some morphological, anatomical and biotechnological investigations, will also be conducted to some of selected palms in order to insure cultivar identity and to evaluate fruit quality. Micropropagation by means of tissue culture technique, beside traditional method of date palm propagation by offshoots, will also be practiced to further produce elite cultivars.

Recycling date palm leaf waste as sustainable alternative for paper production

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Abstract

The UAE has more than 40 million date palms, and about three-quarters of them are grown in the Emirate of Abu Dhabi. Nearly 15 kg of biomass is produced by each date palm annually. Cumulatively, this amounts to between 350-525 million leaves per year, which is a huge biomass. Unfortunately, this biomass is severely underutilized largely because little is known about the composition of the leaves as a material/substrate. Hence, it is largely considered as waste. In order to explore the possibility to use this waste for paper production, we conducted /chemical characterization of the i) date palm leaves and ii) the leaf rachis fronds to assess their constitution. The leaves analysis showed that of the total leaf content, *a-cellulose* content in the inner leaf whorl (ILW), middle leaf whorl (MLW) and outer leaf whorl (OLW) is 41.6%, 46.6%, and 48.1% respectively. The corresponding values for *hemicellulose* from the inner (ILW), middle (MLW) and outer leaf whorl (OLW) are 13.7% 15.4% and 16.03%, respectively, and the *lignin*, an aromatic complex polymer of monolignols which acts as the strong binding material of the fibers in paper pulp is 32.6 %, 34.1 % and 39.1 %, respectively. The leaf rachis analysis from inner, middle and outer fronds of the canopy showed that the *a-cellulose* content is 40.6%, 42.1% and 44.4% in the inner whorl rachis (IWR), middle whorl rachis (MWR) and outer whorl rachis (OWR) fronds, respectively. Next, the *hemicellulose* content for the corresponding fronds is 12.6% (IWR), 13.4% (MWR) and 15.6% (OWR), and the *lignin* content is 31.6%, 33.6% and 36.2%, respectively. Further, the *total extractives*, a key determinant of pulp quality, from leaf blade and rachis together were found to be 8%. Last, mineral analysis was also carried out to precisely characterize the chemical status of the leaf. The overall content analysis of the date palm leaves clearly shows the potential of using the leaf-waste as a source for pulp/paper production. Moreover, compared to other pulp/paper producing natural sources like soft wood, hard wood, straw, bamboo and fir trees, the key pulp indicators in date palm leaves are comparable or better. Current efforts in our laboratory are being directed towards creating different grades of paper from the leaves to assess future commercial prospects of paper production from this abundant yet largely underutilized waste.

Key words: Date palm leaf waste, cellulose, hemicellulose, lignin, pulp, paper production.

Effect of mycorrhiza-associated bacteria on mycorrhization, growth and uptake of mineral nutrition in date palm seedlings.

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Abstract

Soil micro-organisms, used as bio-fertilizers, have a beneficial effect on plant growth and mineral nutrition. But studies made on these bio-fertilizers remain again very limited and even absent for the date palm cultivation in Tunisia. This study was conducted to study the effect of inoculation with spores of the mycorrhizal fungus genera *Glomus* (M) and a range of bacterial strains (Bi) intimately linked at the mycorrhizal interface, taken from rhizosphere of date palm trees in the Djerid region, on date palm seedlings grown in a soil of the region under greenhouse conditions. The results show that several bacterial strains were found with a dominance of 15 strains. In this study, the bacterial strain B14 (*Pseudomonas luteola*) presents a great plasticity to overcome the pedoclimatic constraints of the oases. Increases of 33.3%, 86% and 37% were observed for N, P and K, respectively, from date palm seedlings compared to treatment T. While bacterial strain B4, showed decreases in the order of 19.44%, 14.89% and 23.53% respectively for N, P and K, taken from the date palm seedlings, comparing to treatment M. The results of the analysis showed that in general, regardless of the nature of the biological combination Bi + M or M, the inoculated date palm seedlings were always larger than those for the treatment T (control). From these preliminary results, it appears that the use of biofertilizers (mycorrhizal fungi and mycorrhizal helper bacteria) is a promising approach in organic farming.

Keywords: Mycorrhizal fungi, bacteria, bio-fertilizers, rhizosphere, date palm.

INTRODUCTION

The rhizosphere is the zone of influence of plant roots on the associated microbiota and soil components, characterized by an altered microbial diversity with increased activity and number of microorganisms (Kennedy et al, 1998).The microbial activity in rhizosphere is under direct influence of plant roots, which release organic material, mainly as root exudates. These exudates serve as substrates for the indigenous microorganisms. On the other hand, microorganisms associated with plant roots, both free and symbiotically living, would help the host plant to adapt to stress conditions concerning water and mineral nutrition and soil-borne plant pathogens (Lynch, 1990).

In fact, several rhizobacteria play a major role in enhancing the growth and health of plants. The beneficial effects of rhizobacteria have been attributed towards their ability to promote plant growth by either facilitating resource acquisition (nitrogen, phosphorus and other essential minerals) or modulating plant hormone levels. In addition, several researchers have noted that the establishment and functioning of mycorrhizal symbioses can be positively influenced by some bacterial strains named Mycorrhiza Helper Bacteria (MHB) (Frey-Klett et al, 2007).Consequently, the use of MHB in interaction with indigenous rhizospheric microorganisms (rhizobia and AMF) seems to be a real agro-ecological alternative to chemical fertilization. Hence, the present study was carried out to evaluate the efficiency of some MHB on mycorrhization, mineral nutrition and plant biomass of date palm seedlings cultivated in South-West of Tunisia.

MATERIALS AND METHODS

Plant material and soils used

The material used in this study is the arbuscular mycorrhizal fungal (AMF) spore and mycorrhiza-associated bacteria (MAB) of the host plant: *Phoenix dactilifera L.* (Date palm).

The soils of Djerid region were selected for cultivation of date palm seedlings grown in plastic pots under greenhouse conditions at the regional research center of oasis agriculture. Pots were filled with 3kg sterilized soils of Djerid region. Three replications were prepared for each treatment.

The considered soils studied at all depths are generally characterized by coarse textures, slightly alkaline to alkaline pH values, very low cation exchange capacity (CEC); the percentage of active limestone is moderately high (Table 1).

Inoculation of seedlings with AMF and mycorrhiza-associated bacteria (MAB)

AMF spores were isolated using the wet-sieving (125 and 45 µm) and decanting method described by Gerdemann and Nicolson (1963). Spores and spore clusters were transferred into Petri dishes and counted in three replications under binocular microscope with magnification of 40X and divided in groups in relation to morphological characteristics as shape, size, color, presence of structures like sporiferous saccule, subtending hypha. Although, the same morphotypes of mycorrhizal fungal spores (genus *Glomus)* were isolated in collaboration with Dr. Yolande Dalpé (Eastern cereal and Oilseed Research Centre (ECORC) Ottawa). Approximately 50 spores were isolated and counted for each pot and stored at 4°C for a maximum of two days before pots application.

Mycorrhiza-associated bacteria (MAB) were collected from the rhizosphere of date palm. Samples of soil were suspended in saline solution and serial dilutions were spread on NYDA «Nutrient Yeast Dextrose Agar». The bacterial isolates were characterized by conventional tests on API 20 E gallery and on specific reactive media in collaboration with Environmental Health Laboratory (EHL) Tozeur. For the inoculums preparation the selected MAB strains were inoculated separately in 250 ml flasks containing 100 ml of NYDA broth. The flasks were kept in a growth chamber at 28°C on an orbital shaker at 120 rpm. After 7 days 1 ml of the broth culture was spread on NYDA plates to determine the microbial concentration. Finally, the bacterial cultures were adjusted to a concentration of approximately 10^8 cfu / ml (Murray et al., 2003).

The date palms seeds were surface sterilized by agitation in 0.5% sodium hypochlorite for 5 min, followed by 6 washings with sterile water. Seeds were then germinated in 1% agar water (w/v) plates for 72 h at 28°C. After germination, seedlings were planted in sterilized soils of Djerid region. The experiment includes the following treatments:

1- Control treatment (T): untreated soil
2- M: Spores of the AMF (genus *Glomus*) (50 spores per pot)
3- Bi + M; (Bi: (B1, B2,…, B15)).

One germinated seed was sown in each pot and inoculated directly with 1.5 ml of bacterial culture (10^8 cfu ml^{-1}) grown in NYDA.

Mycorrhizal parameters and plant growth

Plants were snatched 120 days after sowing in greenhouse under controlled conditions. The leaf area was estimated using the equation given by Ahmed and Morsy (1999): Leaf area (cm^2) = 0.70 (length x width) – 1.06. Then, plants were uprooted carefully from the soil and washed with water. Also, shoot weight was measured after oven drying at 62°C for 72 hours.

A part of the root of each plant was collected, cleared and stained as described by Phillips and Haymann (1970) and finally mounted on slides. Quantification of arbuscular mycorrhizal infection of date palm seedlings roots was determined using the magnified line-intersect method of McGonigle et al. (1990).

Plant mineral analysis (N, P, K)

Shoot samples were oven-dried at 68 °C for 48 hours, ground, and passed through a 1 mm sieve. Then, the Kjeldahl method was used to determine total nitrogen (N) after wet digestion with concentrated sulfuric acid. Also, Phosphorus (P) and potassium (K) were performed using the method described by Pauwels et al, (1992).

Statistical analysis

The statistical treatment of results is achieved through the STATISTICA Version 5 software, (Beaux et al. 1991). Analysis of variance of two factors by Fisher's F test is performed to verify the equality of the means of hypothesis risk threshold of 5%. It is supplemented by multiple comparisons of means by the Newman Keuls test when the equality of averages hypothesis is rejected, according to Dagnelie (1986).

RESULTS AND DISCUSSION

Evaluation of plant mycorrhization

On the soil of Djerid region, dual inoculation of plants with spores of the AMF and the majority of mycorrhiza-associated bacteria (MAB) improved the mycorrhization rate in root (MR%) compared to the treatments having single inoculation (plants inoculated with spores of the AMF) (Fig. 1).

Treatment

The results showed that the bacterial strain B14 (*Pseudomonas luteola*) gives the highest value, and there are significant differences for the majority of treatments.

Among these 15 bacterial strains, B4 and B7 (*Stenotrophomonas maltophilia, Chryseobacterium meningosepticum*) decreased significantly arbuscular abundance in the mycorrhizal root cortex (A%) (Fig. 1, 2). Thus, B4 and B7 bacterial strain has a negative impact on mycorrhizal infection. While inoculation with the others bacterial strain promoted the intensity of root cortex colonization (H%, A% and V%). All bacteria studied with the exception of B4 (Stenotrophomonas maltophilia) and B7 (Chryseobacterium meningosepticum) are mycorrhiza helper bacteria.

The experiment showed that there are bacteria that are auxiliaries of mycorhization so-called "mycorrhiza helper bacteria", while, others have inhibitory activity on the development of mycorrhizae. This result matches those found by Garbaye and Bowen (1989) who demonstrated that the rhizosphere microflora could have a positive or negative impact on the mycorrhizal symbiosis depending on the bacterial isolates.

Inoculation effect on plant development

Inoculation of plants with mycorrhiza-associated bacteria (MAB) increased palm seedlings shoot height on the sterilized soils of Djerid region. But statistical analysis shows some stability for all treatments. Similarly, these rhizobacteria improved also date palm seedling leaf area (Table 1). Table.1 Effect of mycorrhiza-associated bacteria inoculations on growth of date palm seedlings. Whereas the decrease in spore density of the mycorrhizal fungus over time proves the germination of these forms of conservation and the appearance of new fungal structures

Nutrient content of plants (N, P and K)

Date palm seedling inoculation with all bacteria studied with the exception of B4 (*Stenotrophomonas maltophilia*) and B7 (*Chryseobacterium meningosepticum*), enhance plants content in potassium and phosphorus (Table 2). Moreover, this increase is accompanied by an improvement in plant nitrogen content. But statistical analysis shows some stability of the nitrogen content for all treatments.

In this study, our interest was to assess date palm seedling growth, under the effect of 15 rhizobacteria estimated as mycorrhiza-associated bacteria (MAB), in interaction with indigenous AMF. The results show that most of bacterial inoculation has a positive effect on nutrient uptake of date palm seedling.

The study on the presence of different mycorrhizal structures in the roots of date palm seedling confirmed the mycorrhizal status of date palm, considered as a mycothrophic species (Zougari et al. 2016). Thus, it was shown that the dual inoculation (AMF and mycorrhiza-associated bacteria (MAB)), with the exception of treatment (B4+M and B7+M), increased the mycorrhization rate of date palm seedling in comparison with single inoculation (M). Furthermore, the *Pseudomonas luteola* had the highest capacity to enhance plant's mycorrhizal infection. This bacterium might be favorable to mycorrhizal infection by producing organic acids and some growth factors such as IAA.

Several studies have reported the ability of pseudomonads to act as mycorrhiza helper bacteria (MHB) (Frey-Klett et al., 2007; Naziret al., 2010). It has been previously demonstrated that some pseudomonds promote the saprophytic growth and root colonization by AMF (Pivato et al., 2009). Indeed, Pseudomonas luteola can positively influence the efficiency of mycorrhization by increasing arbuscular exchange area. Likewise, phosphate solubilizing in the rhizosphere is one of the most important actions of this bacterial strain (Aarab et al., 2015). Thus, they are estimated as phosphate solubilizing bacteria (PSB).

The release of phosphate from insoluble compounds in rhizosphere involves specific enzymatic processes. The phosphate solubilization capacity by these PSB is widely associated to production of low molecular weight organic acids (Qureshi et al., 2012).The released acid chelate the cations (Al, Fe, Ca) bound to the insoluble forms of inorganic phosphate and convert them into soluble forms with the consequent decrease in the pH of the medium (Stevenson 2005). Similarly, the fungal hyphae are capable of releasing the insoluble phosphate by secreting extracellular enzymes (phosphatase, phytase) (Gobat et al., 2003).

Consequently, AMF improve the absorption of phosphorus and other nutrients by plants increasing the contact surface and the explored soil volume (He & Nara, 2007). Experiments have shown that the improvement in the absorption of phosphate is one of the mechanisms by which the AMF may improve the productivity of plant; they contribute to 90% in phosphate uptake by plants (Van der Heijden, 2006).

Ability of plant growth promoting rhizobacteria (PGPR) to affect root architecture is mainly related to their influence in the hormonal balance of plants, especially in the relationship between auxins and cytokinins (Vacheron et al., 2013). These 2 hormones stimulate the elongation of root hairs (Contesto et al., 2008; Galland et al, 2012) and increase number and size of secondary roots (Chamamet al., 2013).

As suggested by Contesto (2008), the inhibitory effect of date palm seedling mycorrhization by B4 (*Stenotrophomonas maltophilia*) and B7 *(Chryseobacterium meningosepticum*) could be explained by the competition with rhizobia for food and other nutrients. These bacteria may also inhibit growth in a given symbiotic relationship, depending upon the nature and concentration of secondary metabolites released in rhizosphere (Contesto et al., 2008).

An important supply of phosphate for plants inoculated with most of mycorrhiza-associated bacteria (MAB), explains also the increase in leaf area and shoot height. Studies have shown that mycorrhization had a significant effect on leaf area and shoot height of date palm (Radi et al., 2014). In addition, Ben Brahim et al. (1996) and Ben Brahim (1996) showed according to their studies of maritime pine seedlings that growth is affected by a phosphorus deficiency and reduction in leaf area. Indeed, the rate of leaf elongation (or the rate of leaf expansion) is the determining factor in reducing the final size of the leaves in case of P deficiency (Chiera et al., 2002).

CONCLUSION

In conclusion, we report that B14 (*Pseudomonas luteola*) in interaction with indigenous AMF have great benefits on mineral nutrition and growth of date palm seedling. Thus interactions between indigenous micro-organisms (AMF and mycorrhiza-associated bacteria (MAB)) can be strongly influenced by these bacteria estimated as MHB. Because of their potential to increase plant nutrition and growth, (B14+M) seem therefore to be the key biofertilizers able to promote the agro-ecological yield of date palm cultivated in the southwest of Tunisia. However, further investigations are needed to explore if this association (B14+M) behave similarly in field studies.

References

Aarab, S., J. Ollero, M. Megías, A. Laglaoui, M. Bakkali and Arakrak, A. 2015. Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An in vitro study. International Journal of Current Microbiology and Applied Sciences. 4(1): 260-269.

Ahmed F.F. and Morsy, M.H. 1999. A new methods for measuring leaf area in different fruit species. Minia J Argic Res & Dev. 19: 97-105

Beaux, M.F., H. Gouet, J.P. Gouet, P. Morghem, G. Philippeau, J. Tranchefort and Verneau M. 1991. Manuel d'utilisation du Logiciel STAT-ITCF.1st ed. Paris. Imprimerie Institut Technique des Céréales et des Fourrages

Ben Brahim, M. 1996. Effets de la nutrition phosphatée sur la croissance et le bilan de carbone des jeunes plants de Pin maritime. PhD, Université Henri Poincaré, Nancy I, France.

Ben Brahim, M., D. Loustau, J.P. Gaudillère and Saur, E. 1996. Effects of phosphate deficiency on hotosynthesis and accumulation of starch and soluble sugars in 1-year-old seedlings of maritime pine (Pinus pinaster Aït.). Ann Sci For. 53: 801 810.

Chamam, A., H. Sanguin,F. Bellvert, G. Meiffren, G.Comte, F. Wisniewski-Dye, C. Bertrand and Prigent-Combaret, C. 2013. Plant secondary metabolite profiling evidences strain-dependent effect in the Azospirillum-Oryza sativaassociation. Phytochemistry. 87: 65-77.

Chiera, J., J.Thomas and Rufty, T. 2002. Leaf initiation and development in soybean under phosphorus stress. J Exp Bot. 53: 473-481.

Contesto, C., G. Desbrosses, C. Lefoulon, G. Béna, F. Borel, M. Galland, L. Gamet, F. Varoquaux and Touraine, B. 2008. Effects of rhizobacterial ACC- deaminase activity on Arabidopsis indicate that ethylene mediates local root reponses to plant growth promoting rhizobacteria. Plant Sci. 175:178-189.

Dagnelie, P. 1986. Théorie et méthodes statistiques. 2nd ed. Gembloux, Belgique : Presses agronomiques

Frey-Klett, P., J.Garbaye and Tarkka, M. 2007. The mycorrhiza helper bacteria revisited. New Phytologist. 176: 22-36.

Galland, M., L. Gamet, F. Varoquaux, B. Touraine and Desbrosses, G. 2012. The ethylene pathway contributes to root hair elongation induced by the beneficial bacteria Phyllobacterium brassicacearum STM196. Plant Science. 190: 74-81.

Garbaye, J. and Bowen, G.D. 1989. Stimulation of mycorrhizal infection of Pinus radiata by some microorganisms associated with the mantle of ectomycorrhizas. New Phytologist 112, 383-388.

Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society.235-244.

Gobat, J.M., M.Aragno and Matthey, W. 2003. Le sol vivant, 2 e Edition. Presses Polytechniques Universitaires Romandes, Lausanne. 568 p.

He, X., and Nara, K. 2007. Element biofortification: can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? Trends in Plant Science. 12: 331-333.

Kennedy. A.C. The rhizosphere and spermosphere. In: Sylvia. D.M.. Fuhrmann. J.J.. Hartel. P.G.. Zuberer. D.A. (eds). (1998). *Principles and applications of soil microbiology*. Prentice Hall. Upper Saddle River. New Jersey. USA. p.389-407.

Lynch. J.M. (1990). Beneficial interactions between micro-organisms and roots. *Biotechnol. Adv.* 8(2).335-346.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, Swan, J.A. 1990. A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501. doi : 10.1111/j.1469-8137.1990.

Murray, P., E. Baron, J. Jorgensen, M.A. Pfaller, Yorken, R.H. 2003. Susceptibility testing methods yeast and filamentous fungi, manual of clinical microbiology 8th ed. Vol. 2 American Society Microbiology press Washington DC.

Nazir, R., J.A.Warmink, H.Boersma andVan Elsas, J.D. 2010. Mechanisms that promote bacterial fitness in fungal- affected soil microhabitats. FEMS Micobiol Ecol.71:169-185.

Pauwels J.M., E. Van Ranst, M. Verloo, Mvondo, Z.A. 1992. Méthodes d'analyses d'éléments majeurs dans la plante. Manuel de laboratoire de pédologie: Méthodes d'analyses des sols et des plantes. Equipement, gestion des stocks de verrerie et produits chimiques. 1st ed. AGCD, Dschang-Bruxelles. Publications agricoles.

Phillips, J.M., and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158-161

Pivato, B., P. Offre, S. Marchelli, B. Barbonaglia, C. Mougel, P. Lemanceau and Berta, G. 2009. Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi and host plant. Mycorrhiza. 19:81-90.

Qureshi, M.A., Z.A.Ahmad, N.Akhtar, A. Iqbal, F. Mujeeb and Shakir, M.A. 2012. Role of phosphate solubilizing bacteria (PSB) in enhancing phosphate availability and promoting cotton growth. The J. of Ani. and Plant Sci.22 (1): 204-210.

Radi, M., H. Hamdali,A.Meddich, L.Ouahmane and Hafidi, M. 2014. The mycorrhizal potential of urban soils in semi-arid zones and tolerance of date palm (Phoenix dactylifera L.) to water deficit. J. Mater. Environ. Sci. 5 (6):1957-1967.

Stevenson, F.J. 2005. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. Wiley, Sons, (ed) New York

Vacheron, J., G.Desbrosses, M-L.Bouffaud, B.Touraine, Y.Moënne-Loccoz, D.Muller, L.Legendre, F.Wisniewski- Dyé and Prigent-Combaret, C. 2013. Plant growth-promoting rhizobacteria and root system functioning. Frontiers in Plant Science. 4:356.

Van der Heijden, M.G.A., R.Streitwolf-Engel, R.Riedl, S.Siegrist, A.Neudecker, K. Ineichen, T.Boller, A.Wiemken and Sanders, I.R. 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure inexperimental grassland. New Phytologist. 172:739-752

Zougari, B., W. Issami, A. Msetra, M. Sanaa, Y. Dalpé and Lounes A.H.S. 2016. Monitoring the evolution of the arbuscular mycorrhizal fungi associated with date palm. Journal of new sciences.31 (12):1822-1831.

Tables

Table I. Chemical and textural characteristics of the soils from Djerid region

Depth (cm)	pH	EC (mmhos/cm)	CEC (mmol (+)/kg)	$CaCO_3$ (%)		Texture class
				Total	Active	
0-20	8.14 ±0.04	4.2 ±0.17	5.2	13.5 ± 1.21	7.0 ±0.08	SCL
20-40	8.22 ±0.08	3.1 ±0.02	4.7	15.2 ±1.17	8.3 ±0.31	SL
40-60	7.43 ±0.01	3.1 ±0.14	4.8	12.4 ±1.24	8.1 ±1.06	SL

SCL: Sandy Cay Loam, SL: Sandy Loam, EC: electrical conductivity, $CaCO_3$: calcium carbonate

Table.1 Effect of mycorrhiza-associated bacteria inoculations on growth of date palm seedlings.

	Shoot height (cm)	Root length (cm)	Leaf area (cm^2)	Spores number /1kg soil	Dry shoot weight (g)
T	14.12 a	16.05 b	6,35 e	0,00	0.70 de
M	16.83 a	21.33 ab	8,48 cd	4,00	1.13 cd
B1+M	16.80 a	20.80 ab	8,11 cd	4,00	0.91 d
B2+M	15.87 a	21.67 ab	7,46 d	3,00	1.17 cd
B3+M	16.50 a	16.59 b	10,88 b	5,00	0.71 de
B4+M	14.38 a	15.85 b	7,49 d	6,00	0.86 d
B5+M	17.07 a	20.27 ab	10,49 b	4,00	1.05 cd
B6+M	15.88 a	18.75 ab	9,22 c	5,00	1.24 c
B7+M	15.50 a	15.79 b	8,98 c	6,00	1.08 cd
B8+M	14.50 a	19.00 ab	7,47 d	5,00	0.74 de
B9+M	16.50 a	21.75 a	11,26 b	3,00	1.14 cd
B10+M	15.50 a	20.67 ab	12,32 ab	4,00	1.10 cd
B11+M	16.73 a	20.83 ab	10,07 c	4,00	1.52 b
B12+M	17.17 a	18.33 b	13,06 a	5,00	1.18 cd
B13+M	11.00 a	20.50 ab	12,74 a	4,00	1.10 cd
B14+M	18.24 a	22.75 a	14,26 a	3,00	1.87 a
B15+M	16.74 a	22.30 a	7,73 d	4,00	1.43 bc

Values in lines followed by different letter differ significantly

Table.2 Effect of mycorrhiza-associated bacteria (MAB) inoculations on uptake (N, P, K) of date palm seedling

Treatment	N (‰)	P (‰)	K (%)
T	0.60 a	0.43 b	0.27 ab
M	0.72 a	0.47 b	0.31 ab
B1+M	0.71 a	0.54 ab	0.37 a
B2+M	0.60 a	0.53 ab	0.37 a
B3+M	0.74 a	0.64 a	0.38 a
B4+M	0.58 a	0.40 b	0.26 ab
B5+M	0.80 a	0.75 a	0.36 a
B6+M	0.60 a	0.62 a	0.34 a
B7+M	0.72 a	0.45 b	0.33 a
B8+M	0.60 a	0.75 a	0.37 a
B9+M	0.72 a	0.78 a	0.36 a
B10+M	0.60 a	0.54 ab	0.33 a
B11+M	0.72 a	0.74 a	0.36 a
B12+M	0.80 a	0.69 a	0.35 a
B13+M	0.60 a	0.64 a	0.34 a
B14+M	0.80 a	0.80 a	0.37 a
B15+M	0.72 a	0.56 ab	0.32 a

Values in lines followed by different letter differ significantly

Figures

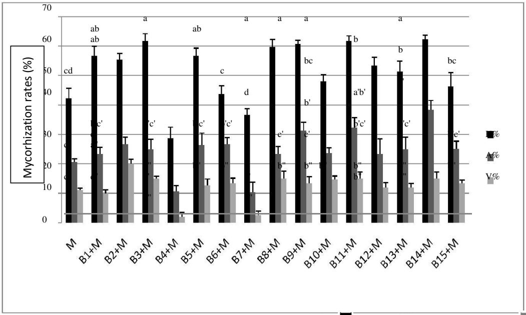

Fig. 1. Effects of treatments on mycorrhization rate; hyphal ( ), arbuscular, ( ) and vesicular ( ) in the roots of date palm seedling.

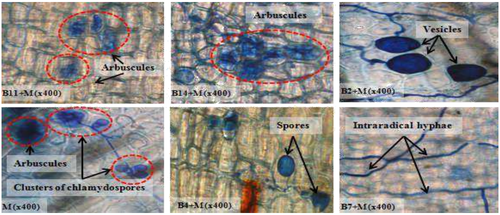

Fig.2. Mycorrhizal infection of date palms seedlings in response to bacterial inoculation

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